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BIOASSAY OF 2-CHLORO-p-PHENYLENEDIAMINE SULFATE FOR POSSIBLE CARCINOGENICITY

CAS No. 61702-44-1

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U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service
National Institutes of Health

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REPORT ON BIOASSAY OF 2-CHLORO-P-PHENYLENEDIAMINE SULFATE FOR POSSIBLE CARCINOGENICITY

Availability

2-Chloro-p-phenylenediamine sulfate (CAS 61702-44-1) has been tested for cancer-causing activity with rats and mice in the Bioassay Program, Division of Cancer Cause and Prevention, National Cancer Institute. A report is available to the public.

Summary: A bioassay for possible carcinogenicity of 2-chloro-p-phenylenediamine sulfate was conducted using Fischer 344 rats and B6C3F1 mice. Applications of the chemical include use as an ingredient of hair dyes. 2-Chloro-p-phenylenediamine sulfate was administered in the feed, at either of two concentrations, to groups of 50 male and 50 female animals of each species.

Under the conditions of this bioassay there was insufficient evidence that dietary administration of 2-chloro-p-phenylenediamine sulfate was carcinogenic to Fischer 344 rats or B6C3F1 mice.

Single copies of the report are available from the Office of Cancer Communications, National Cancer Institute, Building 31, Room 10A21, National Institutes of Health, Bethesda, Maryland 20014.

Dated: October 6, 1978

Director
National Institutes of Health

(Catalogue of Federal Domestic Assistance Program Number 13.393, Cancer Cause and Prevention Research)

BIOASSAY OF

2-CHLORO-p-PHENYLENEDIAMINE SULFATE

FOR POSSIBLE CARCINOGENICITY

Carcinogenesis Testing Program
Division of Cancer Cause and Prevention
National Cancer Institute
National Institutes of Health
Bethesda, Maryland 20014

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REPORT ON THE BIOASSAY OF 2-CHLORO-p-PHENYLENEDIAMINE SULFATE FOR POSSIBLE CARCINOGENICITY

CARCINOGENESIS TESTING PROGRAM
DIVISION OF CANCER CAUSE AND PREVENTION
NATIONAL CANCER INSTITUTE, NATIONAL INSTITUTES OF HEALTH

FOREWORD: This report presents the results of the bioassay of 2-chloro-p-phenylenediamine sulfate conducted for the Carcinogenesis Testing Program, Division of Cancer Cause and Prevention, National Cancer Institute (NCI), National Institutes of Health, Bethesda, Maryland. This is one of a series of experiments designed to determine whether selected chemicals have the capacity to produce cancer in animals. Negative results, in which the test animals do not have a significantly greater incidence of cancer than control animals, do not necessarily mean the test chemical is not a carcinogen because the experiments are conducted under a limited set of circumstances. Positive results demonstrate that the test chemical is carcinogenic for animals under the conditions of the test and indicate a potential risk to man. The actual determination of the risk to man from animal carcinogens requires a wider analysis.

CONTRIBUTORS: This bioassay of 2-chloro-p-phenylenediamine sulfate was conducted by Mason Research Institute, Worcester, Massachusetts, initially under direct contract to the NCI and currently under a subcontract to Tracor Jitco, Inc., prime contractor for the NCI Carcinogenesis Testing Program.

The experimental design was determined by the NCI Project Officers, Dr. J. H. Weisburger (1,2) and Dr. E. K. Weisburger (1). The principal investigators for the contract were Dr. E. Smith (3) and Dr. A. Handler (3). Animal treatment and observation were supervised by Mr. G. Wade (3) and Ms. E. Zepp (3). Chemical analysis was performed by Midwest Research Institute (4) and the analytical results were reviewed by Dr. N. Zimmerman (5).

Histopathologic examinations were performed by Dr. A. S. Krishna Murthy (3) at the Mason Research Institute, and the diagnoses included in this report represent the interpretation of this pathologist. Histopathology findings and reports were reviewed by Dr. R. L. Schueler (6).

Compilation of individual animal survival, pathology, and summary tables was performed by EG&G Mason Research Institute (7); the statistical analysis was performed by Mr. W. W. Belew (5) using methods selected for the Carcinogenesis Testing Program by Dr. J. J. Gart (8).

This report was prepared at METREK, a Division of The MITRE Corporation (5) under the direction of the NCI. Those responsible for this report at METREK are the project coordinator, Dr. L. W. Thomas (5), task leader Dr. M. R. Kornreich (5), senior biologist Ms. P. Walker (5), biochemist Mr. S. C. Drill (5), and technical editor Ms. P. A. Miller (5). The final report was reviewed by members of the participating organizations.

The following other scientists at the National Cancer Institute were responsible for evaluating the bioassay experiment, interpreting the results, and reporting the findings: Dr. K. C. Chu (1), Dr. C. Cueto, Jr. (1), Dr. J. F. Douglas (1), Dr. D. G. Goodman (1), Dr. R. A. Griesemer (1), Dr. H. A. Milman (1), Dr. T. W. Orme (1), Dr. R. A. Squire (1,9), Dr. J. M. Ward (1), and Dr. C. E. Whitmire (1).

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SUMMARY

A biossay for possible carcinogenicity of 2-chloro-p-phenylenediamine sulfate was conducted using Fischer 344 rats and B6C3F1 mice. 2-chloro-p-phenylenediamine sulfate was administered in the feed, at either of two concentrations, to groups of 50 male and 50 female animals of each species. The dietary concentrations used in the chronic bioassay were 0.3 and 0.15 percent for the high and low dose rats, respectively, and 0.6 and 0.3 percent for the high and low dose mice, respectively. Compound administration was for 105 to 107 weeks in rats, 87 weeks in high dose mice, and 104 to 105 weeks in low dose mice. The only groups observed during an untreated period after dosing were the high dose mice, observed for 18 weeks after compound administration ceased. For each species, 20 animals of each sex were placed on test as controls.

There were no significant positive associations between the administered dietary concentrations of 2-chloro-p-phenylenediamine sulfate and mortality for rats of either sex or male mice. There was a significant positive association between dosage and mortality for female mice; however, adequate numbers of animals in all groups survived sufficiently long to be at risk from late-developing tumors.

There were no statistically significant positive associations between dietary exposure to the compound and the incidences of any tumor in rats. There was an increased incidence of transitional-cell hyperplasia of the renal pelvic epithelium in both male and female rats, and transitional-cell tumors of the urinary bladder were present in three dosed rats. These lesions indicated a possible carcinogenic effect, but are not considered as sufficient evidence of carcinogenicity. In mice no tumors occurred in statistically significantly higher incidences in the dosed mice than in controls.

Under the conditions of this bioassay there was insufficient evidence that dietary administration of 2-chloro-p-phenylenediamine sulfate was carcinogenic to Fischer 344 rats or B6C3F1 mice.

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I. INTRODUCTION

2-Chloro-p-phenylenediamine sulfate (NCI No. C03316) is a salt of 2-chloro-p-phenylenediamine and sulfuric acid. It is a component of commercial hair dyes and was selected for bioassay by the National Cancer Institute because of the increased bladder cancer incidence noted among dye manufacturing industry workers (Anthony and Thomas, 1970; Wynder et al., 1963). This elevated cancer risk has been associated with occupational exposure to several classes of compounds, including aromatic amines (Wynder et al., 1963). The widespread exposure to 2-chloro-p-phenylenediamine or its mono- or disulfate salts among hair dye users and the suspected increase in the incidence of bladder cancer among hairdressers (Anthony and Thomas, 1970) were also important factors in its selection for testing.

The Chemical Abstracts Service (CAS) Ninth Collective Index (1977) name for this compound is 2-chloro-1,4-benzenediamine sulfate.* It is also called 3-chloro-4-aminoaniline sulfate; o-chloro-p-phenylenediamine sulfate; and C.I. (Colour Index) Oxidation Base 13A (C.I. 76066). 2-Chloro-p-phenylenediamine is known as Ursol Brown O (C.I. 76065).

2-Chloro-p-phenylenediamine or the corresponding sulfates are primary intermediates in at least 31 commercially available permanent hair dyes (Richardson, 1977). These compounds react with other dye components within the hair shaft to produce permanent hair colors (Corbett and Menkart, 1973).

*The CAS registry number is 61702-44-1.

Filaments and fibers of aromatic polyamides, such as p-aminobenzoyl chloride-2-chloro-p-phenylenediamine-piperazine-terephthaloyl dichloride copolymer (Fujiwara et al., 1974) and poly(2-chloro-p-phenyleneterephthalamide) (Bair and Morgan, 1975) have been produced experimentally using 2-chloro-p-phenylenediamine; however, these polymers do not appear to be used commercially.

All persons whose hair is colored with dyes containing 2-chloro-p-phenylenediamine or the mono- or disulfate salts experience dermal contact and local absorption may occur. Hairdressers may also be exposed through dermal contact. Approximately 40 percent of U.S. women are regular hair dye users, and sales of the permanent type of dyes account for about 75 percent of all hair dye expenditures (Corbett and Menkart, 1973). Portions of 2-chloro-p-phenylenediamine or its sulfate salts which fail to react with the other dye constituents may reach rivers and streams via domestic wastewater.

The potential for exposure to 2-chloro-p-phenylenediamine compounds may also be considerable for workers in the dye and chemical manufacturing industries and for those engaged in research with the various polyamide fibers.

Lowenstein Dyes and Cosmetics, Inc. (undated) reports the following effects of 2-chloro-p-phenylenediamine sulfate exposure: skin irritation, asthmatic symptoms, and eye damage.

II. MATERIALS AND METHODS

A. Chemicals

2-Chloro-p-phenylenediamine sulfate (Figure 1) was purchased from Aldrich Chemical Company, Milwaukee, Wisconsin. Chemical analysis was performed by Midwest Research Institute, Kansas City, Missouri. The manufacturer's specified purity for this chemical was 98 percent. The experimentally determined melting point range was 250° to 262°C, compared to a reference value of 251° to 253°C (Aldrich Chemical Company, Inc., 1977). The wide range suggested the presence of impurities. Thin-layer chromatography utilizing two solvent systems (ethyl acetate: ammonium hydroxide and benzene:1,4-dioxane: ammonium hydroxide), each visualized with ultraviolet light and furfural showed, respectively, one less motile and one nonmotile impurities and two less motile and one nonmotile impurities. Results of elemental analysis were consistent with $ClC_6H_3(NH_2)_2 \cdot H_2SO_4$, the molecular formula for 2-chloro-p-phenylenediamine sulfate. High-pressure liquid chromatography revealed one peak with a tail, again suggesting the presence of impurities. Vapor-phase chromatography showed one homogenous peak.

Throughout this report, the term 2-chloro-p-phenylenediamine sulfate is used to refer to the commercially available product used in this bioassay.

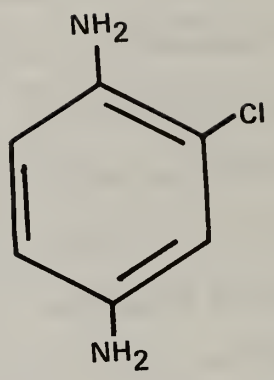


FIGURE 1
CHEMICAL STRUCTURE OF 2-CHLORO-p-PHENYLENEDIAMINE (SULFATE)

B. Dietary Preparation

The basal laboratory diet for both treated and control animals consisted of Wayne Lab-Blox® (Allied Mills, Inc., Chicago, Illinois). 2-Chloro-p-phenylenediamine sulfate was administered to the treated animals as a component of the diet.

The chemical was removed from its container and proper amounts were weighed out under an exhaust hood. The compound was hand-blended in an aluminum bowl with an aliquot of the ground feed. Once visual homogeneity was attained, the mixture was placed in a 6 kg capacity Patterson-Kelley standard model twin-sheel stainless steel V-blender along with the remainder of the feed to be prepared. After 20 minutes of blending, the mixture was placed in double plastic bags and stored in the dark at 4°C. The mixture was prepared once weekly.

C. Animals

Two animals species, rats and mice, were used in the carcinogenicity bioassay. Fischer 344 rats and B6C3F1 mice were obtained through contracts of the Division of Cancer Treatment, National Cancer Institute. All rats and mice were supplied by Charles River Breeding Laboratories, Inc., Wilmington, Massachusetts. Treated and control animals for each species were received in separate shipments.

Upon arrival, a sample of animals was examined for parasites and other signs of disease. All animals were treated with 3.0 gm piperazine adipate per litre of water given ad libitum for three days, followed by three days of tap water and three more days of piperazine

adipate administration. All animals were quarantined by species for 2 weeks prior to initiation of the test. Animals were assigned to groups and distributed among cages so that the average body weight per cage was approximately equal for a given species and sex.

D. Animal Maintenance

All animals were housed by species in rooms having a temperature range of 23° to 34°C. Incoming air was filtered through Tri-Dek® 15/40 denier Dacron® filters (Tri-Dim Filter Corp., Hawthorne, New Jersey) providing six changes of room air per hour. Fluorescent lighting was provided on a 12-hour-daily cycle.

Rats were housed five per cage by sex. During quarantine and for the first 15 months of study, all rats were housed in wire-mesh cages (Fenco Cage Products, Boston, Massachusetts) suspended over newspapers. Newspapers under cages were replaced daily and cages and racks washed weekly. For the remainder of the study, all rats were held in suspended polycarbonate cages (Lab Products, Inc., Garfield, New Jersey) equipped with disposable nonwoven fiber filter sheets. Clean cages and bedding were provided twice weekly. Aspen hardwood chip bedding (American Excelsior Company, Baltimore, Maryland) was used in polycarbonate cages. Stainless steel cage racks (Fenco Cage Products) were cleaned once every 2 weeks.

Mice were housed five per cage by sex in polycarbonate cages fitted with perforated stainless steel lids (Lab Products, Inc.) and nonwoven fiber filter bonnets. Clean cages, lids, and bedding

were provided twice per week. SAN-I-CEL® (Paxton Processing Company, Paxton, Illinois) and Bed-o-Cobs® (The Andersons Cob Division, Maumee, Ohio) corncob bedding were used for the initial 15 months of study. Aspen bedding was used for the remainder of the study. Reusable filter bonnets and pipe racks were sanitized every 2 weeks throughout the study.

Water was available ad libitum for both species from 250 ml water bottles equipped with rubber stoppers and stainless steel sipper tubes. Bottles were replaced twice weekly and, for rats only, water was supplied as needed between changes.

During the period of chemical administration, treated and control animals received treated or untreated Wayne Lab-Blox® meal as appropriate. The food, replenished daily, was supplied in Alpine® aluminum feed cups (Curtin Matheson Scientific, Inc., Woburn, Massachusetts) for the first 15 and 9 months of study for rats and mice, respectively. For the remainder of the bioassay, meal was provided in stainless steel gangstyle food hoppers (Scientific Cages, Inc., Bryan, Texas), in which food was replenished daily.

All rats were housed in a room with other rats receiving diets containing* N-butylurea (592-31-4); 1,5-naphthalenediamine (2243-62-1); N,N-dimethyl-p-nitrosoaniline (138-89-6); 2,5-toluenediamine sulfate (6369-59-1); 2,4-dinitrotoluene (121-14-2); 4-nitroanthranilic acid (619-17-0); N-(1-naphthyl)ethylenediamine dihydrochloride

*CAS registry numbers are given in parentheses.

(1465-25-4); aniline hydrochloride (142-04-1); and p-anisidine hydrochloride (20265-97-8).

All mice were housed in a room with other mice receiving diets containing hydrazobenzene (530-50-7); 2,3,5,6-tetrachloro-4-nitroanisole (2438-88-2); tris(2,3-dibromopropyl)phosphate (126-72-7); and aniline hydrochloride (142-04-1).

E. Selection of Initial Concentrations

In order to establish the maximum tolerated concentrations of 2-chloro-p-phenylenediamine sulfate for administration to treated animals in the chronic studies, subchronic toxicity tests were conducted with both rats and mice.

Animals of each species were distributed among six groups, each consisting of five males and five females. 2-Chloro-p-phenylenediamine sulfate was incorporated into the basal laboratory diet and supplied ad libitum to five of the six groups of each species in concentrations of 3.0, 1.0, 0.3, 0.1, and 0.03 percent. The sixth group of each species served as a control group, receiving only the basal laboratory diet. The dosed dietary preparations were administered for a period of 8 weeks. Individual body weights were recorded weekly throughout the study. Daily food consumption per cage was monitored during the test. At the end of the 8-week period, all survivors were sacrificed and necropsied.

The highest concentration causing no deaths, no compound-related gross abnormalities, and no mean body weight depression in excess of

15 percent relative to controls during the 8-week subchronic test was selected as the high concentration utilized for the rat and mouse chronic bioassays.

At a dietary concentration of 1.0 percent all the male and one of the female rats died. No mortality occurred in rats receiving a dietary concentration of 0.3 percent and mean body weight depressions were 10.4 and 6.4 percent in male and female rats, respectively. A dietary concentration of 0.1 percent 2-chloro-p-phenylenediamine sulfate induced no deaths or mean body weight depression in either males or females. The high concentration selected for administration to rats in the chronic bioassay was 0.3 percent.

At a dietary concentration of 1.0 percent, one male mouse died and mean body weight depression was 3.8 and 14.4 percent for male and female mice, respectively. A dietary concentration of 0.3 percent induced no deaths in either sex, produced no mean body weight depression in males and produced mean body weight depression of 16.1 percent in female mice.

The high concentration selected for administration to mice in the chronic bioassay was 0.6 percent.

F. Experimental Design

The experimental design parameters for the chronic study (species, sex, group size, concentrations administered and duration of treated and untreated observation periods) are summarized in Tables 1 and 2.

TABLE 1

DESIGN SUMMARY FOR FISCHER 344 RATS
2-CHLORO-p-PHENYLENEDIAMINE SULFATE FEEDING EXPERIMENT

	INITIAL GROUP SIZE	2-CHLORO-p-PHENYLENEDIAMINE CONCENTRATION (PERCENT)	OBSERVATION PERIOD	
			TREATED (WEEKS)	UNTREATED (WEEKS)
MALE				
CONTROL	20	0	0	107
LOW DOSE	50	0.15	105	0
HIGH DOSE	50	0.30	106	0
FEMALE				
CONTROL	20	0	0	108
LOW DOSE	50	0.15	106	0
HIGH DOSE	50	0.30	107	0

TABLE 2

DESIGN SUMMARY FOR B6C3F1 MICE
2-CHLORO-p-PHENYLENEDIAMINE SULFATE FEEDING EXPERIMENT

	INITIAL GROUP SIZE	2-CHLORO-p-PHENYLENEDIAMINE CONCENTRATION (PERCENT)	OBSERVATION PERIOD	
			TREATED (WEEKS)	UNTREATED (WEEKS)
MALE				
CONTROL	20	0	0	107
LOW DOSE	50	0.3	104	0
HIGH DOSE	50	0.6 0	87	18
FEMALE				
CONTROL	20	0	0	107
LOW DOSE	50	0.3	105	0
HIGH DOSE	50	0.6 0	87	18

The treated and control rats were all approximately 7 weeks old at the time the test was initiated. The initial dietary concentrations of 2-chloro-p-phenylenediamine sulfate were 0.3 and 0.15 percent. Throughout this report the rats receiving the former concentration are referred to as the high dose groups and those receiving the latter concentration are referred to as the low dose groups. Treated rats were supplied with dosed feed for up to a total of 107 weeks.

The treated and control mice were all approximately 7 weeks old at the time the test was initiated. The initial dietary concentrations of 2-chloro-p-phenylenediamine sulfate were 0.6 and 0.3 percent. Throughout this report mice receiving the former concentration are referred to as the high dose groups and those receiving the latter concentration are referred to as the low dose groups. High dose mice were supplied with dosed feed for a total of 87 weeks, followed by an 18-week untreated observation period, due to excessive mortality. Low dose mice were supplied with dosed feed for up to a total of 105 weeks.

G. Clinical and Histopathologic Examinations

Animals were weighed immediately prior to initiation of the experiment. Body weights were recorded twice weekly for the first 12 weeks of the study and at monthly intervals thereafter. Food consumption, for two cages from each group, was monitored for seven consecutive days once a month for the first nine months of the

bioassay and for three consecutive days each month thereafter. From the first day, all animals were inspected twice daily for mortality. The presence of tissue masses and lesions was determined by monthly observation and palpation of each animal.

A necropsy was performed on each animal regardless of whether it died, was killed when moribund, or was sacrificed at the end of the bioassay. The animals were euthanized by carbon dioxide inhalation, and were immediately necropsied. The histopathologic examination consisted of gross and microscopic examination of major tissues, organs, and gross lesions taken from sacrificed animals and, whenever possible, from animals found dead.

Tissues were preserved in 10 percent buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin prior to microscopic examination. An occasional section was subjected to special staining techniques for more definitive diagnosis.

Slides were prepared from the following tissues: subcutaneous tissue, lungs and bronchi, trachea, bone marrow, spleen, lymph nodes, thymus, heart, salivary gland, liver, gallbladder (mice), pancreas, esophagus, stomach, small intestine, large intestine, kidney, urinary bladder, pituitary, adrenal, thyroid, parathyroid, testis, prostate, brain, uterus, mammary gland, and ovary.

A few tissues were not examined for some animals, particularly for those that died early. Also, some animals were missing, cannibalized, or judged to be in such an advanced state of autolysis as to

preclude histopathologic interpretation. Thus, the number of animals for which particular organs, tissues, or lesions were examined microscopically varies and does not necessarily represent the number of animals that were placed on experiment in each group.

H. Data Recording and Statistical Analyses

Pertinent data on this experiment have been recorded in an automatic data processing system, the Carcinogenesis Bioassay Data System (Linhart et al., 1974). The data elements include descriptive information on the chemicals, animals, experimental design, clinical observations, survival, body weight, and individual pathologic results, as recommended by the International Union Against Cancer (Berenblum, 1969). Data tables were generated for verification of data transcription and for statistical review.

These data were analyzed using the statistical techniques described in this section. Those analyses of the experimental results that bear on the possibility of carcinogenicity are discussed in the statistical narrative sections.

Probabilities of survival were estimated by the product-limit procedure of Kaplan and Meier (1958) and are presented in this report in the form of graphs. Animals were statistically censored as of the time that they died of other than natural causes or were found to be missing; animals dying from natural causes were not statistically censored. Statistical analyses for a possible dose-related effect on survival used the method of Cox (1972) when testing two groups for

equality and used Tarone's (1975) extensions of Cox's methods when testing a dose-related trend. One-tailed P-values have been reported for all tests except the departure from linearity test, which is only reported when its two-tailed P-value is less than 0.05.

The incidence of neoplastic or nonneoplastic lesions has been given as the ratio of the number of animals bearing such lesions at a specific anatomic site (numerator) to the number of animals in which that site was examined (denominator). In most instances, the denominators included only those animals for which that site was examined histologically. However, when macroscopic examination was required to detect lesions prior to histologic sampling (e.g., skin or mammary tumors), or when lesions could have appeared at multiple sites (e.g., lymphomas), the denominators consist of the numbers of animals necropsied.

The purpose of the statistical analyses of tumor incidence is to determine whether animals receiving the test chemical developed a significantly higher proportion of tumors than did the control animals. As a part of these analyses, the one-tailed Fisher exact test (Cox, 1970, pp. 48-52) was used to compare the tumor incidence of a control group to that of a group of treated animals at each dose level. When results for a number of treated groups, k, are compared simultaneously with those for a control group, a correction to ensure an overall significance level of 0.05 may be made. The Bonferroni inequality (Miller, 1966, pp. 6-10) requires that the P-value for any comparison

be less than or equal to 0.05/k. In cases where this correction was used, it is discussed in the narrative section. It is not, however, presented in the tables, where the Fisher exact P-values are shown.

The Cochran-Armitage test for linear trend in proportions, with continuity correction (Armitage, 1971, pp. 362-365), was also used when appropriate. Under the assumption of a linear trend, this test determined if the slope of the dose-response curve is different from zero at the one-tailed 0.05 level of significance. Unless otherwise noted, the direction of the significant trend was a positive dose relationship. This method also provides a two-tailed test of departure from linear trend.

A time-adjusted analysis was applied when numerous early deaths resulted from causes that were not associated with the formation of tumors. In this analysis, deaths that occurred before the first tumor was observed were excluded by basing the statistical tests on animals that survived at least 52 weeks, unless a tumor was found at the anatomic site of interest before week 52. When such an early tumor was found, comparisons were based exclusively on animals that survived at least as long as the animal in which the first tumor was found. Once this reduced set of data was obtained, the standard procedures for analyses of the incidence of tumors (Fisher exact tests, Cochran-Armitage tests, etc.) were followed.

When appropriate, life-table methods were used to analyze the incidence of tumors. Curves of the proportions surviving without an

observed tumor were computed as in Saffiotti et al. (1972). The week during which animals died naturally or were sacrificed was entered as the time point of tumor observation. Cox's methods of comparing these curves were used for two groups; Tarone's extension to testing for linear trend was used for three groups. The statistical tests for the incidence of tumors which used life-table methods were one-tailed and, unless otherwise noted, in the direction of a positive dose relationship. Significant departures from linearity ($P < 0.05$, two-tailed test) were also noted.

The approximate 95 percent confidence interval for the relative risk of each dosed group compared to its control was calculated from the exact interval on the odds ratio (Gart, 1971). The relative risk is defined as p_t/p_c where p_t is the true binomial probability of the incidence of a specific type of tumor in a treated group of animals and p_c is the true probability of the spontaneous incidence of the same type of tumor in a control group. The hypothesis of equality between the true proportion of a specific tumor in a treated group and the proportion in a control group corresponds to a relative risk of unity. Values in excess of unity represent the condition of a larger proportion in the treated group than in the control.

The lower and upper limits of the confidence interval of the relative risk have been included in the tables of statistical analyses. The interpretation of the limits is that in approximately 95 percent of a large number of identical experiments, the true ratio

of the risk in a treated group of animals to that in a control group would be within the interval calculated from the experiment. When the lower limit of the confidence interval is greater than one, it can be inferred that a statistically significant result (a $P < 0.025$ one-tailed test when the control incidence is not zero, $P < 0.050$ when the control incidence is zero) has occurred. When the lower limit is less than unity but the upper limit is greater than unity, the lower limit indicates the absence of a significant result while the upper limit indicates that there is a theoretical possibility of the induction of tumors by the test chemical which could not be detected under the conditions of this test.

III. CHRONIC TESTING RESULTS: RATS

A. Body Weights and Clinical Observations

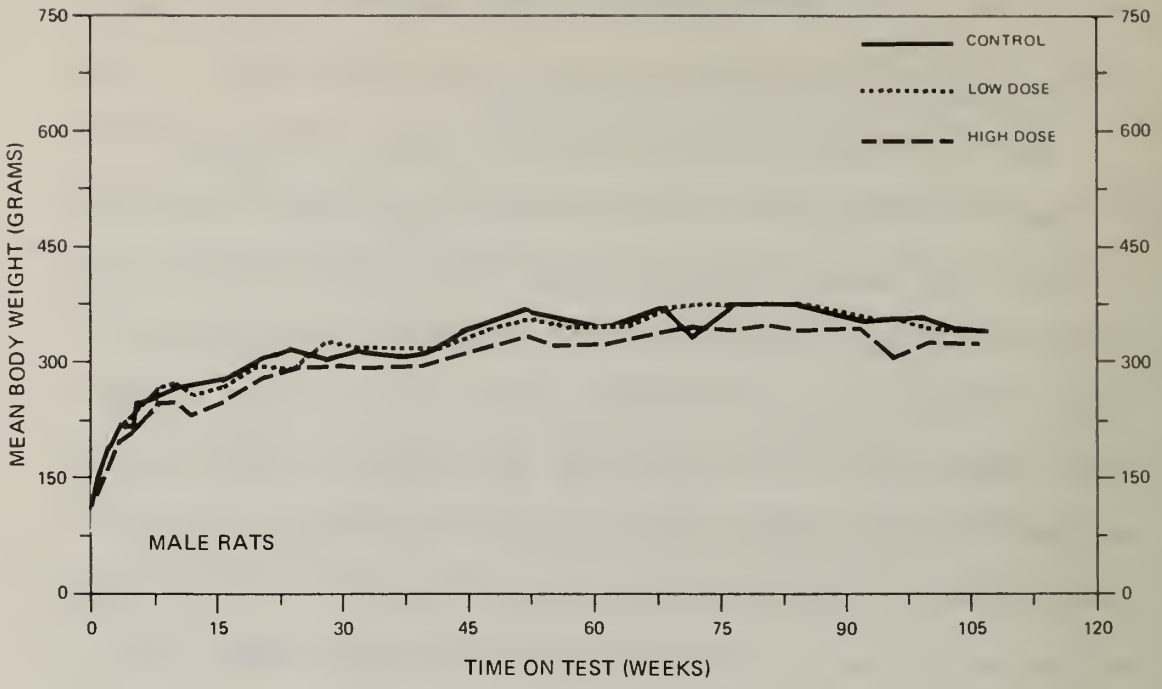
Mean body weight depression was only slight for both male and female treated groups (Figure 2). Eye discoloration, swelling, reddening, and crustation were observed in one high dose male, two low dose males, two high dose females, and four low dose females. Alopecia occurred in one high dose female and one low dose female. Subcutaneous masses were recorded for three high dose males, two low dose males, two high dose females, four low dose females, and two control females. Abdominal distention was observed in one high dose male and general pallor was observed in one low dose female. No other clinical abnormalities were reported.

B. Survival

The estimated probabilities of survival for male and female rats in the control and 2-chloro-p-phenylenediamine sulfate-dosed groups are shown in Figure 3. For male and female rats the Tarone test did not indicate a significant association between dosage and mortality.

Adequate numbers of males were at risk from late-developing tumors, as 80 percent (40/50) of the high dose, 94 percent (47/50) of the low dose, and 90 percent (18/20) of the control group survived on test until the end of the study.

Adequate numbers of females were at risk from late-developing tumors as 94 percent (47/50) of the high dose, 86 percent (43/50) of



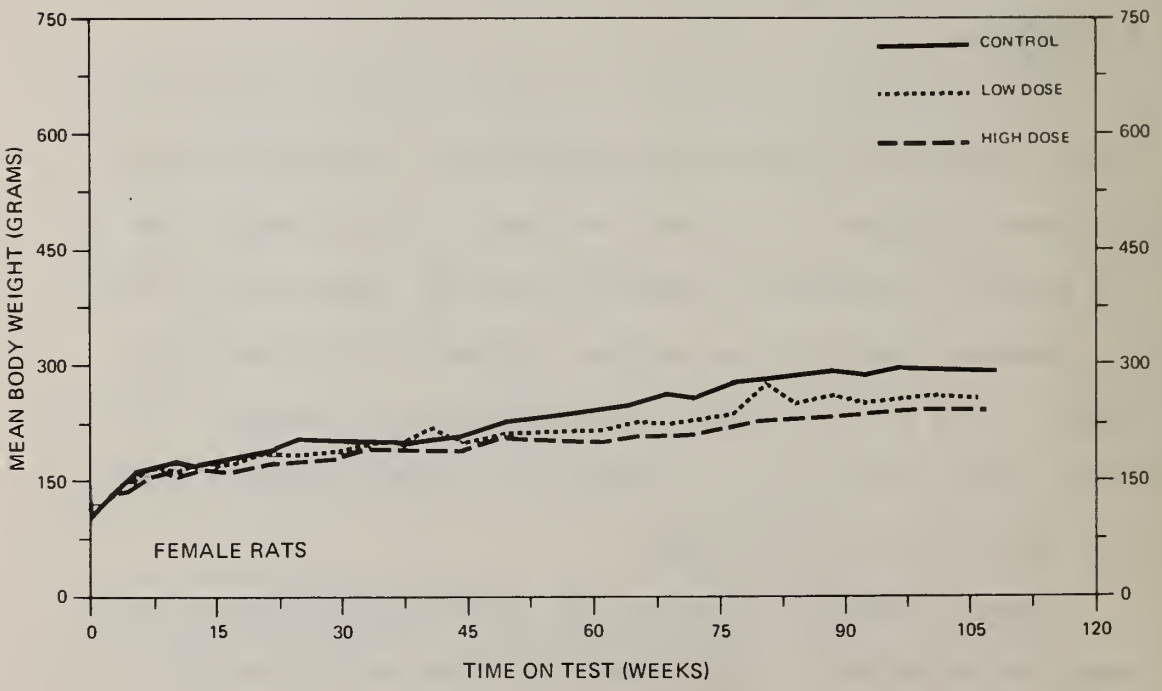
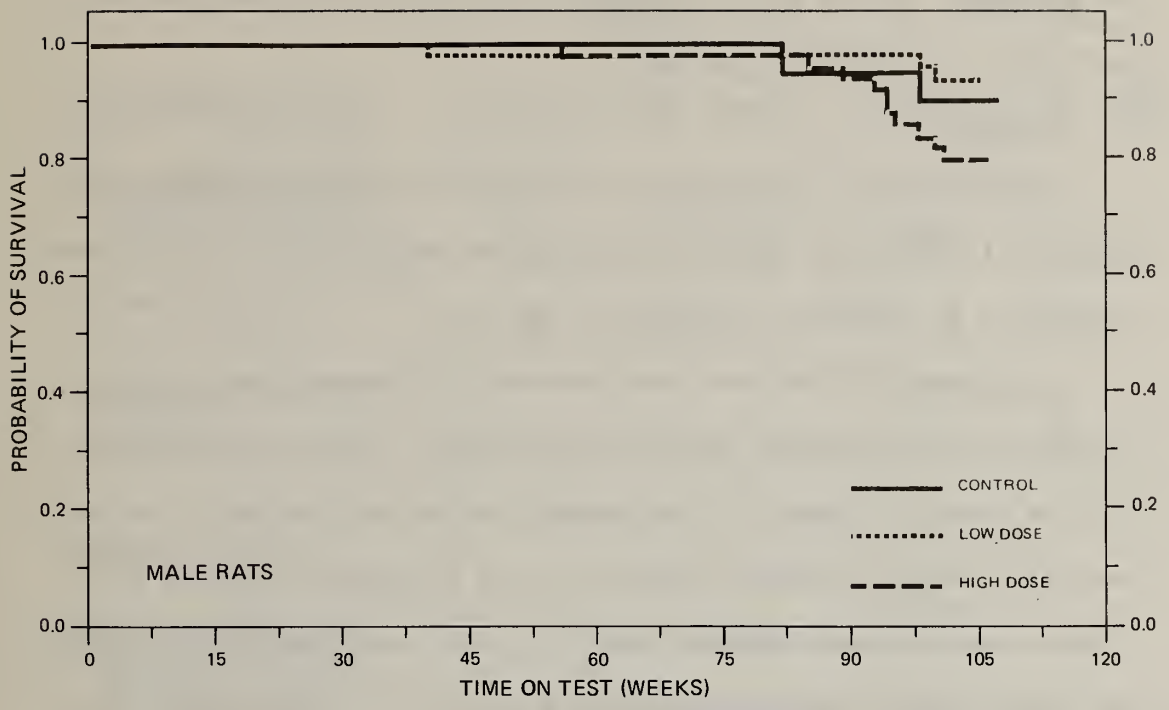


FIGURE 2
GROWTH CURVES FOR 2-CHLORO-p-PHENYLENEDIAMINE SULFATE CHRONIC STUDY RATS



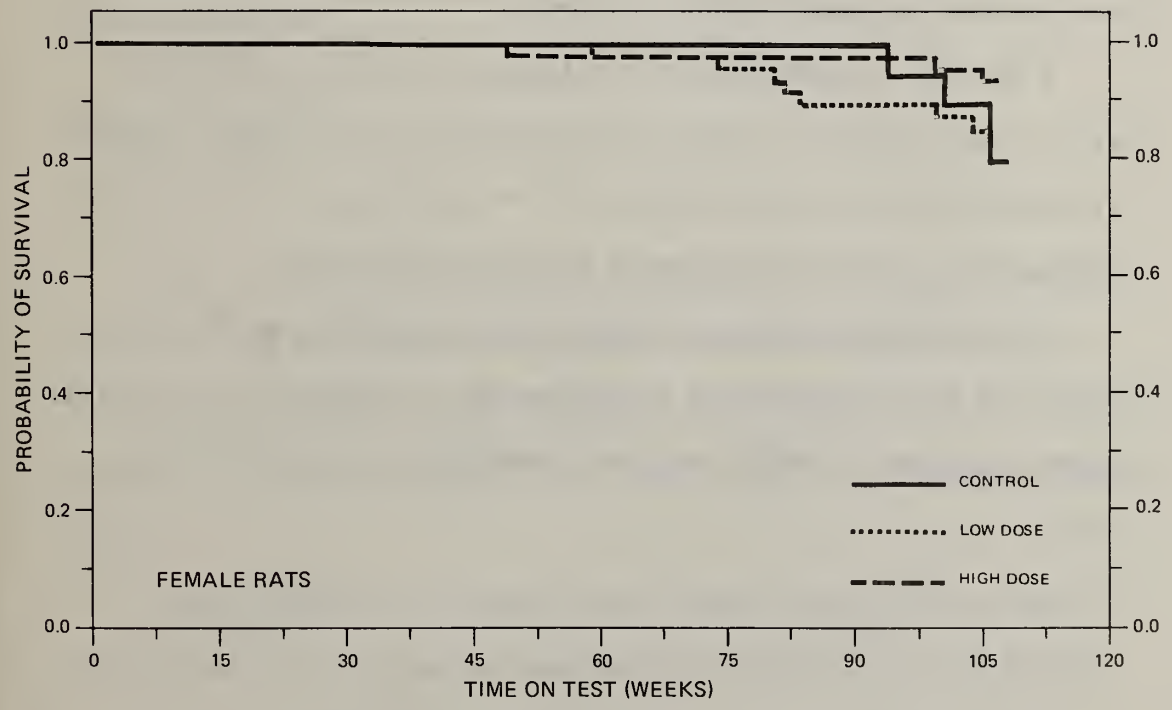


FIGURE 3
SURVIVAL COMPARISONS OF 2-CHLORO-p-PHENYLENEDIAMINE SULFATE CHRONIC STUDY RATS

the low dose, and 80 percent (16/20) of the control group survived on test until the end of the study.

C. Pathology

Histopathologic findings on neoplasms in rats are summarized in Appendix A (Tables A1 and A2); findings on nonneoplastic lesions are summarized in Appendix C (Tables C1 and C2).

A variety of neoplasms were observed with approximately equal frequency in the control and treated animals. There were instances in this study, as noted in the summary tables, of neoplastic lesions occurring only in treated animals or with increased frequency in treated animals when compared with the controls. However, the nature and incidences of these neoplasms are similar to those known to occur spontaneously in aged Fischer 344 rats.

A variety of degenerative, inflammatory, and proliferative lesions were observed in both the treated and control rats. Except for transitional-cell hyperplasia of the renal pelvis, none of the nonneoplastic lesions appeared to be compound-related.

The incidences of transitional-cell hyperplasia of the renal pelvis and transitional-cell papilloma and carcinoma of the urinary system occurring in these rats are summarized in the following table.

	MALES			FEMALES		
KIDNEY/RENAL PELVIS	Control	Low Dose	High Dose	Control	Low Dose	High Dose
No. of Animals with Kidneys Examined Histopathologically	(20)	(49)	(50)	(20)	(48)	(49)
Transitional-Cell Hyperplasia	0	17	30	0	14	8
Transitional-Cell Carcinoma	0	1	0	0	0	0
URINARY BLADDER						
No. of Animals with Urinary Bladders Examined Histopathologically	(18)	(48)	(47)	(20)	(48)	(48)
Transitional-Cell Papilloma	0	1	0	0	0	0
Transitional-Cell Carcinoma	0	0	1	0	1	0

Transitional-cell hyperplasia appeared to be dose-related in male rats and was characterized by an increased cellularity and papillary formation. Mitotic figures were found in areas of hyperplasia. In a few animals, Brunn's nests were cystic and contained blood.

Transitional-cell tumors of the urinary bladder were found in three treated rats (two low dose and one high dose). The bladder epithelium was not hyperplastic in any of the animals examined.

Based upon the findings of this histopathologic examination, 2-chloro-p-phenylenediamine sulfate does not appear to be carcinogenic to Fischer 344 rats.

D. Statistical Analyses of Results

The results of the statistical analyses of tumor incidence in rats are summarized in Tables 3 and 4. The analysis is included for every type of malignant tumor in either sex where at least two such tumors were observed in at least one of the control or 2-chloro-p-phenylenediamine sulfate-dosed groups and where such tumors were observed in at least 5 percent of the group.

There were no tests in either male or female rats indicating a positive association between chemical administration and tumor incidence. Based upon these statistical results, there was no convincing evidence of the carcinogenicity of this compound in rats.

For female rats the Cochran-Armitage test indicated a significant ($P = 0.043$) negative association between dosage and the combined incidence of leukemia or malignant lymphomas. The Fisher exact tests, however, were not significant. Similarly, the Cochran-Armitage test indicated a significant ($P = 0.026$) negative association between dosage and the incidence of islet-cell adenomas of the pancreatic islets, but the Fisher exact tests were not significant.

For female rats the possibility of a negative association between dosage and incidence was noted for endometrial stromal polyps and for pituitary adenomas NOS. It must be noted, however, that the

TABLE 3

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT
SPECIFIC SITES IN MALE RATS TREATED WITH 2-CHLORO-p-PHENYLENEDIAMINE SULFATE[a]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Lung: Alveolar/Bronchiolar Carcinoma or Alveolar/Bronchiolar Adenoma[b]	0/20(0.00)	3/49(0.06)	2/50(0.04)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.255	0.123
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	105	106
Hematopoietic System: Leukemia or Malignant Lymphoma[b]	1/20(0.05)	6/49(0.12)	4/50(0.08)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	2.449	1.600
Lower Limit	---	0.332	0.175
Upper Limit	---	110.166	77.169
Weeks to First Observed Tumor	107	98	85
Liver: Hepatocellular Carcinoma[b]	0/20(0.00)	3/49(0.06)	0/50(0.00)
P Values[c]	N.S.	N.S.	N.S.
Departure from Linear Trend[e]	P = 0.044	---	---
Relative Risk (Control)[d]	---	Infinite	---
Lower Limit	---	0.255	---
Upper Limit	---	Infinite	---
Weeks to First Observed Tumor	---	105	---

TABLE 3 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Liver: Hepatocellular Carcinoma or Neoplastic Nodule[b]	0/20(0.00)	5/49(0.10)	3/50(0.06)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.536	0.250
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	98	56
Pituitary: Adenoma NOS[b]	3/17(0.18)	9/45(0.20)	7/45(0.16)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.133	0.881
Lower Limit	---	0.336	0.237
Upper Limit	---	5.984	4.872
Weeks to First Observed Tumor	107	105	106
Adrenal: Pheochromocytoma[b]	4/20(0.20)	4/48(0.08)	6/50(0.12)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.417	0.600
Lower Limit	---	0.088	0.147
Upper Limit	---	2.063	2.385
Weeks to First Observed Tumor	107	105	101

TABLE 3 (CONCLUDED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Thyroid: C-Cell Adenoma or C-Cell Carcinoma[b]	0/18(0.00)	3/43(0.07)	1/49(0.02)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.264	0.020
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	105	106

[a]Treated groups received doses of 0.15 or 0.30 percent in feed.

[b]Number of tumor-bearing animals/number of animals examined at site (proportion).

[c]The probability level for the Cochran-Armitage test is given beneath the incidence of tumors in the control group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. The probability level for the Fisher exact test for the comparison of a treated group with the control group is given beneath the incidence of tumors in the treated group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. For both Cochran-Armitage and Fisher exact tests a negative designation (N) indicates a lower incidence in the treated group(s) than in the control group.

[d]The 95% confidence interval on the relative risk of the treated group to the control group.

[e]The probability level of the test for departure from linear trend is given beneath the control group when $P < 0.05$.

TABLE 4

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT
SPECIFIC SITES IN FEMALE RATS TREATED WITH 2-CHLORO-p-PHENYLENEDIAMINE SULFATE[a]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Hematopoietic System: Leukemia or Malignant Lymphoma[b]	3/20(0.15)	2/49(0.04)	1/49(0.02)
P Values[c]	P = 0.043(N)	N.S.	N.S.
Relative Risk (Control)[d]	---	0.272	0.136
Lower Limit	---	0.025	0.003
Upper Limit	---	2.233	1.599
Weeks to First Observed Tumor	101	74	105
Pituitary: Adenoma NOS[b]	14/20(0.70)	12/42(0.29)	13/46(0.28)
P Values[c]	P = 0.004(N)	P = 0.002(N)	P = 0.002(N)
Departure from Linear Trend[e]	P = 0.034	---	---
Relative Risk (Control)[d]	---	0.408	0.404
Lower Limit	---	0.246	0.248
Upper Limit	---	0.770	0.753
Weeks to First Observed Tumor	94	82	100
Adrenal: Pheochromocytoma[b]	2/20(0.10)	1/47(0.02)	1/49(0.02)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.213	0.204
Lower Limit	---	0.004	0.004
Upper Limit	---	3.909	3.754
Weeks to First Observed Tumor	107	74	107

TABLE 4 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Thyroid: Follicular-Cell Adenoma or Follicular-Cell Carcinoma[b]	0/20(0.00)	1/44(0.02)	3/39(0.08)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.025	0.321
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	106	106
Pancreatic Islets: Islet-Cell Adenoma[b]	2/20(0.10)	0/48(0.00)	0/48(0.00)
P Values[c]	P = 0.026(N)	N.S.	N.S.
Departure from Linear Trend[e]	P = 0.048	---	---
Relative Risk (Control)[d]	---	0.000	0.000
Lower Limit	---	0.000	0.000
Upper Limit	---	1.400	1.400
Weeks to First Observed Tumor	107	---	---
Mammary Gland: Fibroadenoma[b]	3/20(0.15)	1/49(0.02)	2/49(0.04)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.136	0.272
Lower Limit	---	0.003	0.025
Upper Limit	---	1.599	2.233
Weeks to First Observed Tumor	106	106	107

TABLE 4 (CONCLUDED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Uterus: Endometrial Stromal Polyp[b]	4/20(0.20)	6/48(0.13)	0/49(0.00)
P Values[c]	P = 0.004(N)	N.S.	P = 0.006(N)
Relative Risk (Control)[d]	---	0.625	0.000
Lower Limit	---	0.171	0.000
Upper Limit	---	2.764	0.435
Weeks to First Observed Tumor	94	105	---

[a]Treated groups received doses of 0.15 or 0.30 percent in feed.

[b]Number of tumor-bearing animals/number of animals examined at site (proportion).

[c]The probability level for the Cochran-Armitage test is given beneath the incidence of tumors in the control group when P < 0.05; otherwise, not significant (N.S.) is indicated. The probability level for the Fisher exact test for the comparison of a treated group with the control group is given beneath the incidence of tumors in the treated group when P < 0.05; otherwise, not significant (N.S.) is indicated. For both Cochran-Armitage and Fisher exact tests a negative designation (N) indicates a lower incidence in the treated group(s) than in the control group.

[d]The 95% confidence interval on the relative risk of the treated group to the control group.

[e]The probability level of the test for departure from linear trend is given beneath the control group when P < 0.05.

incidence of pituitary adenomas in the control group (70 percent or 14/20) was somewhat higher than expected based upon the 36 percent (90/249) observed in the historical data on untreated Fischer 344 female rats maintained by this laboratory for the NCI Carcinogenesis Testing Program.

To provide additional insight into the possible carcinogenicity of this compound, 95 percent confidence intervals on the relative risk have been estimated and entered in the tables based upon the observed tumor incidence rates. In many of the intervals shown in Tables 3 and 4, the value one is included; this indicates the absence of statistically significant results. It should also be noted that many of the confidence intervals have an upper limit greater than one, indicating the theoretical possibility of tumor induction in rats by 2-chloro-p-phenylenediamine sulfate that could not be established under the conditions of this test.

IV. CHRONIC TESTING RESULTS: MICE

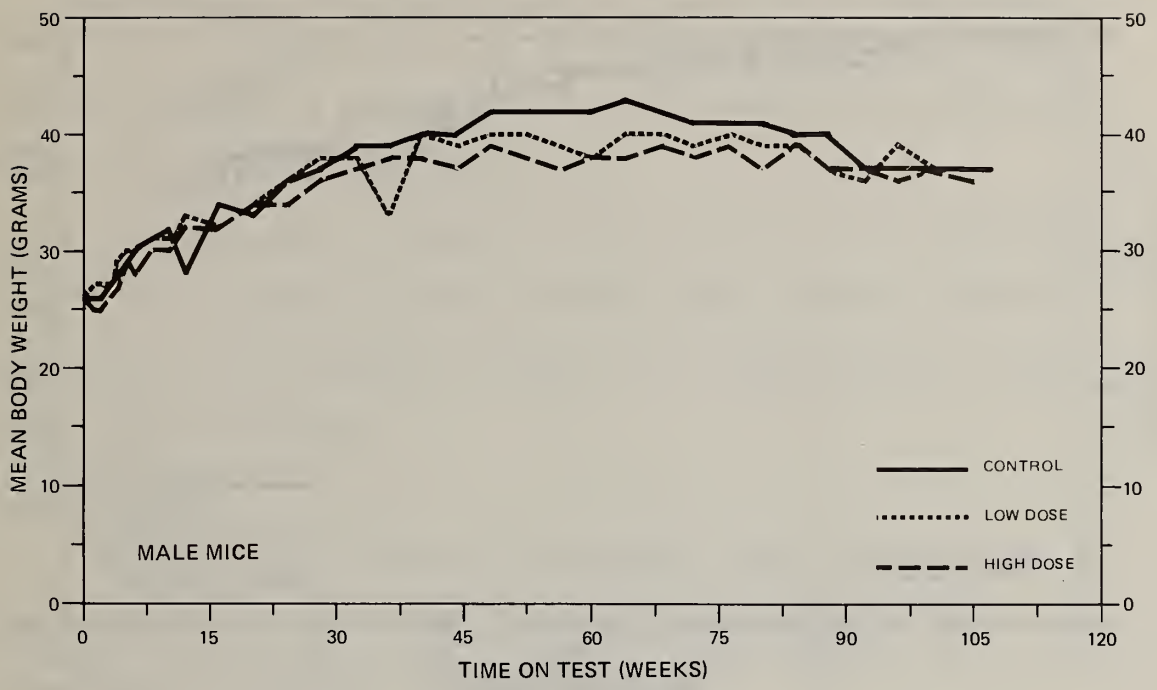
A. Body Weights and Clinical Observations

Mean body weight depression was only slight for both male and female treated mice (Figure 4). Fluctuations in the growth curve may be due to mortality; as the size of the group diminishes, the mean body weight may be subject to wide variations.

Alopecia was recorded for 24 high dose males, 48 low dose males, 15 control males, 8 high dose females, 6 low dose females, and 11 control females. Eye discoloration, swelling, and infection developed in one high dose male, one low dose male, one high dose female, and one control female. Posterior ataxia was reported in one high dose female. Palpable abdominal masses were observed in three low dose females, two control females, and one control male. Subcutaneous masses were reported in one high dose male and two control females. Lightening of fur color was observed in four low dose females. No other clinical abnormalities were reported.

B. Survival

The estimated probabilities of survival for male and female mice in the control and 2-chloro-p-phenylenediamine sulfate-dosed groups are shown in Figure 5. For male mice the Tarone test did not indicate a significant association between dosage and mortality. For female mice the Tarone test indicated a significant ($P < 0.001$) positive association between dosage and mortality.



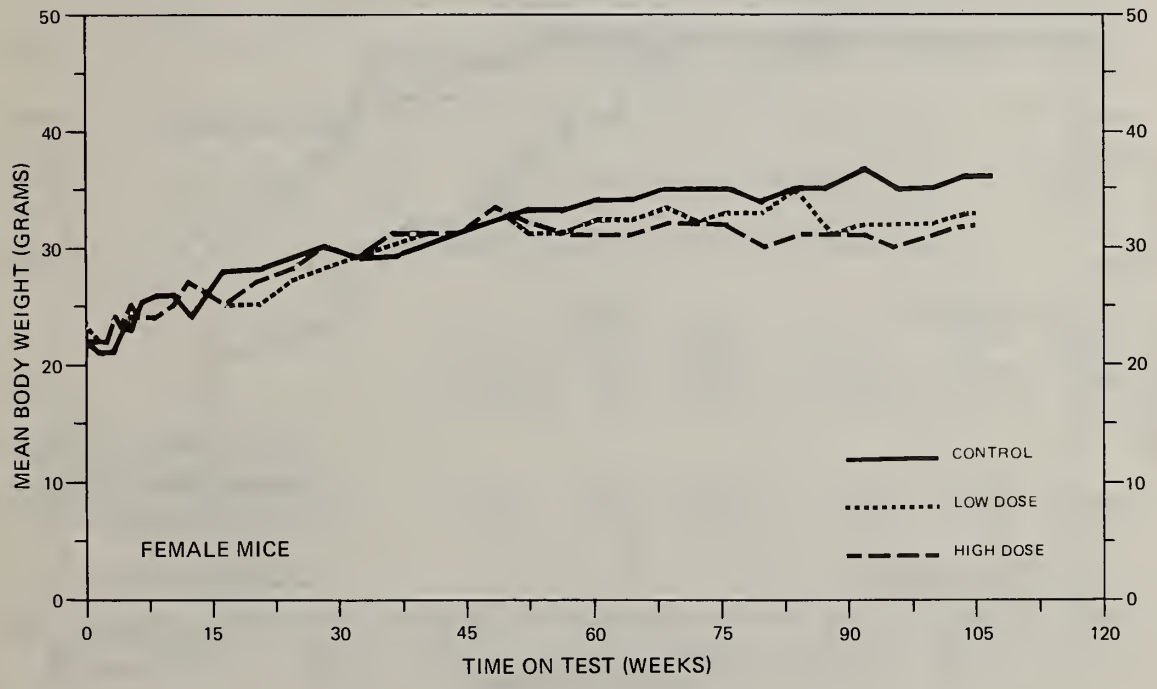


FIGURE 4
GROWTH CURVES FOR 2-CHLORO-p-PHENYLENEDIAMINE SULFATE CHRONIC STUDY MICE

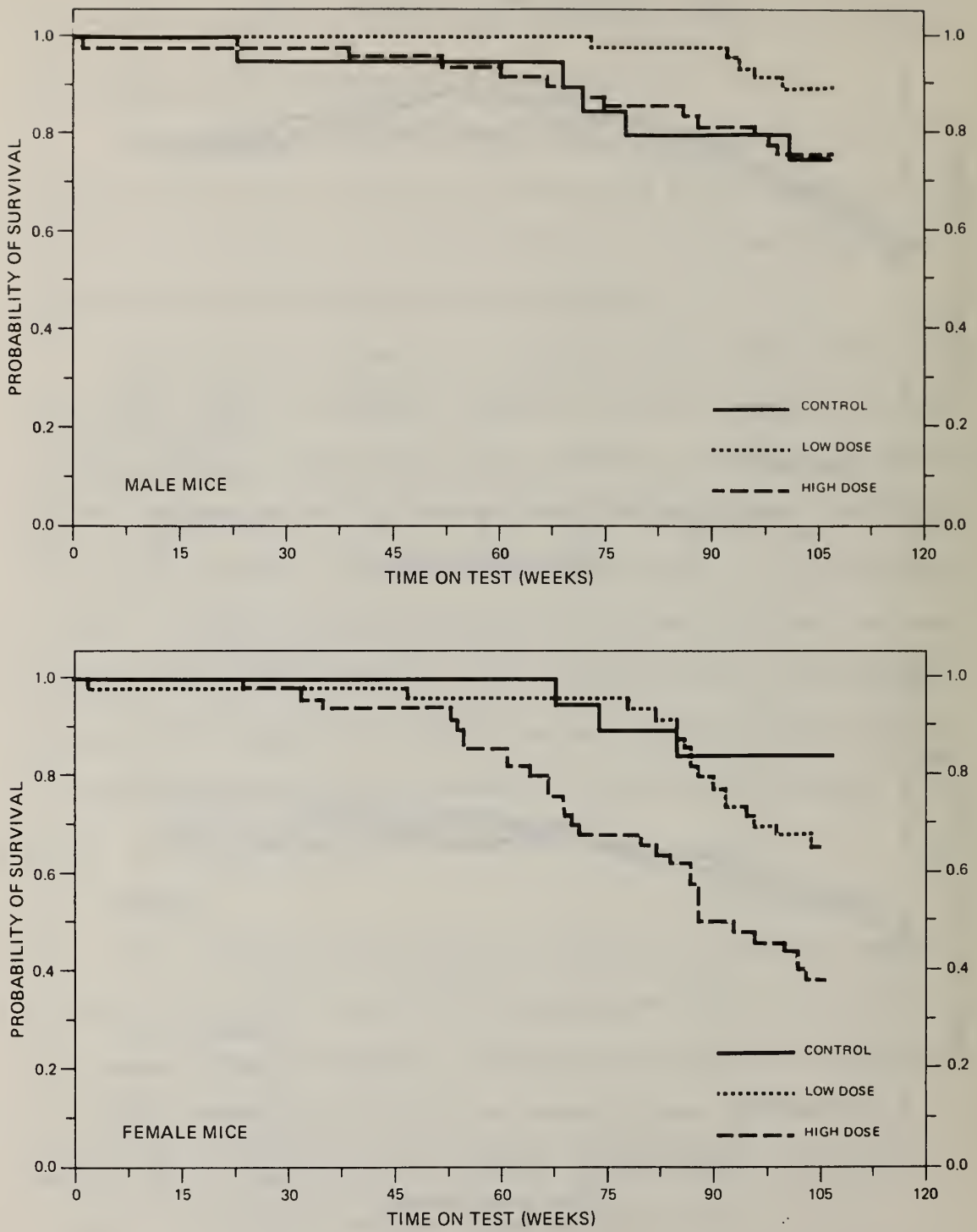


FIGURE 5
SURVIVAL COMPARISONS OF 2-CHLORO-p-PHENYLENEDIAMINE SULFATE CHRONIC STUDY MICE

Adequate numbers of males were at risk from late-developing tumors, as 74 percent (37/50) of the high dose, 90 percent (45/50) of the low dose, and 75 percent (15/20) of the control group survived on test until the end of the study.

Adequate numbers of female mice were at risk from late-developing tumors as 50 percent (25/50) of the high dose, 80 percent (40/50) of the low dose, and 80 percent (16/20) of the control group survived on test at least 90 weeks.

C. Pathology

Histopathologic findings on neoplasms in mice are summarized in Appendix B (Tables B1 and B2); findings on nonneoplastic lesions are summarized in Appendix D (Tables D1 and D2).

A variety of neoplasms were observed in both treated and control mice in this study. There was an increased incidence of proliferative hepatocellular lesions observed in the treated mice of both sexes when compared with control mice. The proliferative hepatocellular lesions observed in this study are summarized in the following table.

	MALES			FEMALES		
	Control	Low Dose	High Dose	Control	Low Dose	High Dose
No. of Animals with Livers Examined Histopathologically	(17)	(49)	(46)	(19)	(49)	(44)
Focal Hyperplasia	0	1	1	0	1	1
Hepatocellular Adenoma	0	4	10	0	3	5
Hepatocellular Carcinoma	4	8	10	2	2	4

A localized increase in the number and size of hepatocytes was considered to be focal hyperplasia. Hepatocellular adenoma was a circumscribed mass involving a few lobules and compressing surrounding parenchyma. Hepatocytes were large with eosinophilic cytoplasm; in a few animals the cytoplasm was vacuolated. Nuclei were vesicular, and there were a few mitotic figures. Hepatocellular carcinoma had involved a part or an entire lobe of the liver. The lobular architecture was distorted and there was marked pleomorphism of hepatocytes. Nuclei were vesicular, and mitotic figures were numerous. Areas of necrosis and mineralization were seen in some of the carcinomas.

In two treated male mice, nodules of markedly basophilic cells were found in conjunction with hepatocellular carcinoma. The nodules were continuous with the carcinoma; no demarcation was apparent. The nodules appeared to be well-vascularized. Many of the cells were cuboidal, and a few were fusiform cells as well. The cytoplasm of these cells was basophilic. Nuclei were vesicular and mitotic figures, numerous. These tumors were considered to be undifferentiated hepatocellular carcinomas, some of which resembled hepatoblastoma.

A variety of inflammatory and degenerative lesions which commonly occur in aging mice of this strain were seen. These nonneoplastic lesions were not considered to be compound-induced.

Based upon the findings of this histopathologic examination, the dietary administration of 2-chloro-p-phenylenediamine sulfate was associated with an increased incidence of proliferative hepatocellular

lesions in the compound treated mice when compared with the untreated control mice.

D. Statistical Analyses of Results

The results of the statistical analyses of tumor incidence in mice are summarized in Tables 5 and 6. The analysis is included for every type of malignant tumor in either sex where at least two such tumors were observed in at least one of the control or 2-chloro-p-phenylenediamine sulfate-dosed groups and where such tumors were observed in at least 5 percent of the group.

For male mice the Cochran-Armitage test indicated a significant ($P = 0.038$) positive association between dosage and the combined incidence of hepatocellular carcinomas or hepatocellular adenomas. The Fisher exact tests, however, were not significant.

For females a significant negative association between dosage and the incidence of thyroid follicular-cell neoplasms was observed, but the Fisher exact tests were not significant. For males the combined incidence of hemangiosarcomas or hemangiomas was significantly ($P = 0.003$) lower in the low dose group than in the control; however, the high dose comparison and the Cochran-Armitage test were not significant.

No other statistical tests were significant for either males or females under the Bonferroni criterion. Based on these statistical results, there was no conclusive evidence of the carcinogenicity of this compound in male or female mice.

TABLE 5

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT
SPECIFIC SITES IN MALE MICE TREATED WITH 2-CHLORO-p-PHENYLENEDIAMINE SULFATE[a]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Lung: Alveolar/Bronchiolar Carcinoma[b]	3/17(0.18)	1/50(0.02)	4/48(0.08)
P Values[c]	N.S.	P = 0.047(N)	N.S.
Departure from Linear Trend[e]	P = 0.030	---	---
Relative Risk (Control)[d]	---	0.113	0.472
Lower Limit	---	0.002	0.092
Upper Limit	---	1.326	2.997
Weeks to First Observed Tumor	107	104	105
Lung: Alveolar/Bronchiolar Carcinoma or Alveolar/Bronchiolar Adenoma[b]	3/17(0.18)	7/50(0.14)	6/48(0.13)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.793	0.708
Lower Limit	---	0.213	0.177
Upper Limit	---	4.407	4.056
Weeks to First Observed Tumor	107	104	88
Hematopoietic System: Leukemia or Malignant Lymphoma[b]	3/17(0.18)	6/50(0.12)	8/50(0.16)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.680	0.907
Lower Limit	---	0.170	0.257
Upper Limit	---	3.900	4.911
Weeks to First Observed Tumor	78	104	88

TABLE 5 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Liver: Hepatocellular Carcinoma[b]	4/17(0.24)	8/49(0.16)	10/46(0.22)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.694	0.924
Lower Limit	---	0.223	0.322
Upper Limit	---	2.860	3.639
Weeks to First Observed Tumor	107	104	105
Liver: Hepatocellular Carcinoma or Hepatocellular Adenoma[b]	4/17(0.24)	12/49(0.24)	20/46(0.43)
P Values[c]	P = 0.038	N.S.	N.S.
Relative Risk (Control)[d]	---	1.041	1.848
Lower Limit	---	0.383	0.761
Upper Limit	---	3.994	6.559
Weeks to First Observed Tumor	107	104	105
Circulatory System: Hemangioma or Hemangiosarcoma[b]	4/17(0.24)	0/50(0.00)	4/46(0.09)
P Values[c]	N.S.	P = 0.003(N)	N.S.
Departure from Linear Trend[e]	P = 0.002	---	---
Relative Risk (Control)[d]	---	0.000	0.370
Lower Limit	---	0.000	0.080
Upper Limit	---	0.361	1.812
Weeks to First Observed Tumor	72	---	96

TABLE 5 (CONCLUDED)

[a]Treated groups received doses of 0.30 or 0.60 percent in feed.

[b]Number of tumor-bearing animals/number of animals examined at site (proportion).

[c]The probability level for the Cochran-Armitage test is given beneath the incidence of tumors in the control group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. The probability level for the Fisher exact test for the comparison of a treated group with the control group is given beneath the incidence of tumors in the treated group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. For both Cochran-Armitage and Fisher exact tests a negative designation (N) indicates a lower incidence in the treated group(s) than in the control group.

[e]The probability level of the test for departure from linear trend is given beneath the control group when $P < 0.05$.

TABLE 6

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT
SPECIFIC SITES IN FEMALE MICE TREATED WITH 2-CHLORO-p-PHENYLENEDIAMINE SULFATE[a]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Lung: Alveolar/Bronchiolar Carcinoma or Alveolar/Bronchiolar Adenoma[b]	0/19(0.00)	3/49(0.06)	2/44(0.05)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.243	0.133
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	104	105
Hematopoietic System: Leukemia or Malignant Lymphoma[b]	8/19(0.42)	8/49(0.16)	8/44(0.18)
P Values[c]	N.S.	P = 0.030(N)	P = 0.048(N)
Relative Risk (Control)[d]	---	0.388	0.432
Lower Limit	---	0.158	0.177
Upper Limit	---	1.034	1.145
Weeks to First Observed Tumor	74	78	54
Liver: Hepatocellular Carcinoma[b]	2/19(0.11)	2/49(0.04)	4/44(0.09)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.388	0.864
Lower Limit	---	0.031	0.139
Upper Limit	---	5.108	9.058
Weeks to First Observed Tumor	107	104	105

TABLE 6 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Liver: Hepatocellular Carcinoma or Hepatocellular Adenoma[b]	2/19(0.11)	5/49(0.10)	9/44(0.20)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.969	1.943
Lower Limit	---	0.180	0.464
Upper Limit	---	9.685	17.429
Weeks to First Observed Tumor	107	104	105
Pituitary: Adenoma NOS[b]	1/15(0.07)	5/35(0.14)	1/24(0.04)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	2.143	0.625
Lower Limit	---	0.277	0.009
Upper Limit	---	98.143	47.035
Weeks to First Observed Tumor	107	88	105
Thyroid: Follicular-Cell Adenoma or Follicular-Cell Carcinoma[b]	2/17(0.12)	1/42(0.02)	0/38(0.00)
P Values[c]	P = 0.041(N)	N.S.	N.S.
Relative Risk (Control)[d]	---	0.202	0.000
Lower Limit	---	0.004	0.000
Upper Limit	---	3.695	1.491
Weeks to First Observed Tumor	107	99	---

TABLE 6 (CONCLUDED)

[a]Treated groups received doses of 0.30 or 0.60 percent in feed.

[b]Number of tumor-bearing animals/number of animals examined at site (proportion).

[c]The probability level for the Cochran-Armitage test is given beneath the incidence of tumors in the control group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. The probability level for the Fisher exact test for the comparison of a treated group with the control group is given beneath the incidence of tumors in the treated group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. For both Cochran-Armitage and Fisher exact tests a negative designation (N) indicates a lower incidence in the treated group(s) than in the control group.

[d]The 95% confidence interval on the relative risk of the treated group to the control group.

To provide additional insight into the possible carcinogenicity of this compound, 95 percent confidence intervals on the relative risk have been estimated and entered in the tables based upon the observed tumor incidence rates. In many of the intervals shown in Tables 5 and 6, the value one is included; this indicates the absence of statistically significant results. It should also be noted that many of the confidence intervals have an upper limit greater than one, indicating the theoretical possibility of tumor induction in mice by 2-chloro-p-phenylenediamine sulfate that could not be established under the conditions of this test.

V. DISCUSSION

There were no significant positive associations between the administered dietary concentration of 2-chloro-p-phenylenediamine sulfate and mortality for rats of either sex or for male mice. There was a significant positive association between dosage and mortality for female mice; however, adequate numbers of animals in all groups survived sufficiently long to be at risk from late-developing tumors.

Although slight mean group body weight depression was observed in treated rats of both sexes when compared to controls, thereby indicating an observable effect of compound administration, there were no statistically significant positive associations between dietary exposure to 2-chloro-p-phenylenediamine sulfate and the incidences of any tumor. The increased incidence of transitional-cell hyperplasia of the renal pelvic epithelium in both male and female rats and the presence of transitional-cell tumors of the urinary bladder in three dosed rats is suggestive of, but not considered as sufficient evidence of, carcinogenicity. The suggestion of carcinogenicity is supported by the absence of these lesions in the concurrent controls and by the historical record of the low incidence of bladder tumors (0/250 males and 1/249 females) in control Fischer 344 rats at this laboratory.

A variety of proliferative hepatocellular lesions were observed in the mice in this bioassay. When those male mice having either hepatocellular carcinomas or hepatocellular adenomas were combined and the resulting tumor incidences were statistically analyzed, there

was a significant positive association between the dietary concentration of the chemical and the incidences. The Fisher exact tests were not, however, supportive. There were no other tumors in mice for which a significant positive association could be established between dosage and incidence. There were also no other tumors occurring in significantly higher incidences in the treated mice than in controls.

Under the conditions of this bioassay there was no convincing evidence that dietary administration of 2-chloro-p-phenylenediamine sulfate was carcinogenic in Fischer 344 rats or B6C3F1 mice.

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APPENDIX A

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN RATS TREATED WITH 2-CHLORO-p-PHENYLENEDIAMINE SULFATE

TABLE A1
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS
TREATED WITH 2-CHLORO-p-PHENYLENEDIAMINE SULFATE

	CONTROL (UNTR) 01-0355	LOW DOSE 01-0340	HIGH DOSE 01-0345
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	49	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	49	50
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(20)	(49)	(50)
FIBROMA	1 (5%)		2 (4%)
RESPIRATORY SYSTEM			
#LUNG	(20)	(49)	(50)
ALVEOLAR/BRONCHIOLAR ADENOMA		1 (2%)	
ALVEOLAR/BRONCHIOLAR CARCINOMA		2 (4%)	2 (4%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(49)	(50)
MALIGNANT LYMPHOMA, NOS			2 (4%)
UNDIFFERENTIATED LEUKEMIA			1 (2%)
MYELOMONOCYTIC LEUKEMIA	1 (5%)	5 (10%)	1 (2%)
#SPLEEN	(20)	(49)	(50)
MYELOMONOCYTIC LEUKEMIA		1 (2%)	
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(20)	(49)	(50)
NEOPLASTIC NODULE		2 (4%)	3 (6%)
HEPATOCELLULAR CARCINOMA		3 (6%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A1 (CONTINUED)

	CONTROL (UNTR) 01-0355	LOW DOSE 01-0340	HIGH DOSE 01-0345
URINARY SYSTEM			
#KIDNEY/PELVIS	(20)	(49)	(50)
TRANSITIONAL-CELL CARCINOMA		1 (2%)	
#URINARY BLADDER	(18)	(48)	(47)
TRANSITIONAL-CELL PAPILLOMA		1 (2%)	
TRANSITIONAL-CELL CARCINOMA			1 (2%)
ENDOCRINE SYSTEM			
#PITUITARY	(17)	(45)	(45)
ADENOMA, NOS	3 (18%)	9 (20%)	7 (16%)
#ADRENAL	(20)	(48)	(50)
PHEOCHROMOCYTOMA	4 (20%)	4 (8%)	6 (12%)
#THYROID	(18)	(43)	(49)
C-CELL ADENOMA		1 (2%)	
C-CELL CARCINOMA		2 (5%)	1 (2%)
#PANCREATIC ISLETS	(18)	(46)	(46)
ISLET-CELL ADENOMA	1 (6%)	1 (2%)	1 (2%)
ISLET-CELL CARCINOMA		1 (2%)	1 (2%)
REPRODUCTIVE SYSTEM			
#TESTIS	(19)	(48)	(50)
INTERSTITIAL-CELL TUMOR	17 (89%)	47 (98%)	48 (96%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*ZYMBAL'S GLAND	(20)	(49)	(50)
CARCINOMA,NOS			1 (2%)
CARCINOSARCOMA			1 (2%)
MUSCULOSKELETAL SYSTEM			
NONE			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A1 (CONCLUDED)

	CONTROL (UNTR) 01-0355	LOW DOSE 01-0340	HIGH DOSE 01-0345
BODY CAVITIES			
*BODY CAVITIES	(20)	(49)	(50)
MESOTHELIOMA, NOS	1 (5%)	1 (2%)	1 (2%)
NEUROFIBROMA			1 (2%)
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	2	2	3
MORIBUND SACRIFICE		1	7
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	18	47	40
ANIMAL MISSING			
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	18	49	50
TOTAL PRIMARY TUMORS	28	82	80
TOTAL ANIMALS WITH BENIGN TUMORS	18	48	48
TOTAL BENIGN TUMORS	26	64	65
TOTAL ANIMALS WITH MALIGNANT TUMORS	1	13	11
TOTAL MALIGNANT TUMORS	1	15	11
TOTAL ANIMALS WITH SECONDARY TUMORS#			
TOTAL SECONDARY TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN- BENIGN OR MALIGNANT	1	3	4
TOTAL UNCERTAIN TUMORS	1	3	4
TOTAL ANIMALS WITH TUMORS UNCERTAIN- PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS
# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

TABLE A2
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS
TREATED WITH 2-CHLORO-p-PHENYLENEDIAMINE SULFATE

	CONTROL (UNTR) 02-0355	LOW DOSE 02-0340	HIGH DOSE 02-0345
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	49	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	49	49
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(20)	(49)	(49)
FIBROMA	1 (5%)	1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(20)	(49)	(49)
SQUAMOUS CELL CARCINOMA, METASTA	1 (5%)		
ADENOCARCINOMA, NOS, METASTATIC	1 (5%)		
ALVEOLAR/BRONCHIOLAR ADENOMA	1 (5%)	1 (2%)	1 (2%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(49)	(49)
MALIGNANT LYMPHOMA, NOS			1 (2%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (2%)	
MYELOMONOCYTIC LEUKEMIA	3 (15%)	1 (2%)	
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(20)	(49)	(49)
NEOPLASTIC NODULE		1 (2%)	1 (2%)
#COLON	(20)	(47)	(49)
MUCINOUS ADENOCARCINOMA		1 (2%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A2 (CONTINUED)

	CONTROL (UNTR) 02-0355	LOW DOSE 02-0340	HIGH DOSE 02-0345
URINARY SYSTEM			
#KIDNEY	(20)	(48)	(49)
ADENOCARCINOMA, NOS, METASTATIC	1 (5%)		
#URINARY BLADDER	(20)	(48)	(48)
TRANSITIONAL-CELL CARCINOMA		1 (2%)	
ENDOCRINE SYSTEM			
#PITUITARY	(20)	(42)	(46)
ADENOMA, NOS	14 (70%)	12 (29%)	13 (28%)
#ADRENAL	(20)	(47)	(49)
PHEOCHROMOCYTOMA	2 (10%)	1 (2%)	1 (2%)
#THYROID	(20)	(44)	(39)
FOLLICULAR-CELL ADENOMA		1 (2%)	
FOLLICULAR-CELL CARCINOMA			3 (8%)
C-CELL ADENOMA	1 (5%)	1 (2%)	
C-CELL CARCINOMA		1 (2%)	
#PANCREATIC ISLETS	(20)	(48)	(48)
ISLET-CELL ADENOMA	2 (10%)		
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(49)	(49)
ADENOMA, NOS		1 (2%)	1 (2%)
ADENOCARCINOMA, NOS	1 (5%)		
FIBROADENOMA	3 (15%)	1 (2%)	2 (4%)
*CLITORAL GLAND	(20)	(49)	(49)
ADENOMA, NOS		1 (2%)	
#UTERUS	(20)	(48)	(49)
ADENOCARCINOMA, NOS	1 (5%)		2 (4%)
ENDOMETRIAL STROMAL POLYP	4 (20%)	6 (13%)	
#OVARY	(20)	(48)	(49)
GRANULOSA-CELL TUMOR		1 (2%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A2 (CONTINUED)

	CONTROL(UNTR) 02-0355	LOW DOSE 02-0340	HIGH DOSE 02-0345
NERVOUS SYSTEM			
#BRAIN	(19)	(48)	(49)
ASTROCYTOMA		1 (2%)	
SPECIAL SENSE ORGANS			
*HARDERIAN GLAND	(20)	(49)	(49)
CARCINOMA,NOS	1 (5%)		
*ZYMBAL'S GLAND	(20)	(49)	(49)
SQUAMOUS CELL CARCINOMA	1 (5%)		
MUSCULOSKELETAL SYSTEM			
*PELVIC BONES	(20)	(49)	(49)
OSTEOSARCOMA	1 (5%)		
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@		5	1
MORIBUND SACRIFICE	4	2	2
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	16	43	47
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A2 (CONCLUDED)

	CONTROL (UNTR) 02-0355	LOW DOSE 02-0340	HIGH DOSE 02-0345
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	17	26	19
TOTAL PRIMARY TUMORS	36	34	25
TOTAL ANIMALS WITH BENIGN TUMORS	17	21	16
TOTAL BENIGN TUMORS	28	26	18
TOTAL ANIMALS WITH MALIGNANT TUMORS	5	6	5
TOTAL MALIGNANT TUMORS	8	6	6
TOTAL ANIMALS WITH SECONDARY TUMORS#	2		
TOTAL SECONDARY TUMORS	3		
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT		2	1
TOTAL UNCERTAIN TUMORS		2	1
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS
\# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

APPENDIX B

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MICE TREATED WITH 2-CHLORO-p-PHENYLENEDIAMINE SULFATE

TABLE B1
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE
TREATED WITH 2-CHLORO-p-PHENYLENEDIAMINE SULFATE

	CONTROL (UNTR) 05-0355	LOW DOSE 05-0345	HIGH DOSE 05-0350
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING	2		
ANIMALS NECROPSIED	17	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	17	50	48
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(17)	(50)	(48)
ALVEOLAR/BRONCHIOLAR ADENOMA		6 (12%)	2 (4%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	3 (18%)	1 (2%)	4 (8%)
PHEOCHROMOCYTOMA, METASTATIC		1 (2%)	
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(17)	(50)	(50)
MALIGNANT LYMPHOMA, NOS	2 (12%)	1 (2%)	3 (6%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (6%)	1 (2%)	2 (4%)
#SPLEEN	(16)	(50)	(45)
HEMANGIOMA	1 (6%)		1 (2%)
HEMANGIOSARCOMA	1 (6%)		1 (2%)
MALIGNANT LYMPHOMA, NOS			1 (2%)
#LYMPH NODE	(14)	(46)	(42)
HEMANGIOMA	1 (7%)		
#MESENTERIC L. NODE	(14)	(46)	(42)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		4 (9%)	2 (5%)
CIRCULATORY SYSTEM			
NONE			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE B1 (CONTINUED)

	CONTROL (UNTR) 05-0355	LOW DOSE 05-0345	HIGH DOSE 05-0350
DIGESTIVE SYSTEM			
#LIVER	(17)	(49)	(46)
HEPATOCELLULAR ADENOMA		4 (8%)	10 (22%)
HEPATOCELLULAR CARCINOMA	4 (24%)	8 (16%)	10 (22%)
HEMANGIOMA	1 (6%)		1 (2%)
HEMANGIOSARCOMA	1 (6%)		2 (4%)
#ESOPHAGUS	(16)	(47)	(42)
BASAL-CELL CARCINOMA			1 (2%)
URINARY SYSTEM			
#KIDNEY	(16)	(50)	(47)
TUBULAR-CELL ADENOMA			1 (2%)
ENDOCRINE SYSTEM			
#ADRENAL	(16)	(49)	(46)
PHEOCHROMOCYTOMA, MALIGNANT		1 (2%)	
REPRODUCTIVE SYSTEM			
#TESTIS	(17)	(49)	(47)
SEMINOMA/DYSGERMINOMA		1 (2%)	
EMBRYONAL CARCINOMA	1 (6%)		
NERVOUS SYSTEM			
#BRAIN	(17)	(50)	(45)
MENINGIOMA	1 (6%)		
SPECIAL SENSE ORGANS			
*HARDERIAN GLAND	(17)	(50)	(50)
ADENOMA, NOS		2 (4%)	
MUSCULOSKELETAL SYSTEM			
NONE			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B1 (CONCLUDED)

	CONTROL (UNTR) 05-0355	LOW DOSE 05-0345	HIGH DOSE 05-0350
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	4	4	9
MORIBUND SACRIFICE	1	1	4
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	13	45	37
ANIMAL MISSING	2		
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	12	21	30
TOTAL PRIMARY TUMORS	17	29	41
TOTAL ANIMALS WITH BENIGN TUMORS	3	10	14
TOTAL BENIGN TUMORS	3	12	15
TOTAL ANIMALS WITH MALIGNANT TUMORS	10	14	24
TOTAL MALIGNANT TUMORS	14	17	26
TOTAL ANIMALS WITH SECONDARY TUMORS#		1	
TOTAL SECONDARY TUMORS		1	
TOTAL ANIMALS WITH TUMORS UNCERTAIN- BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN- PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			
* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS			
# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN			

TABLE B2
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE
TREATED WITH 2-CHLORO-p-PHENYLENEDIAMINE SULFATE

	CONTROL (UNTR) 06-0355	LOW DOSE 06-0345	HIGH DOSE 06-0350
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING	1		
ANIMALS NECROPSIED	19	49	44
ANIMALS EXAMINED HISTOPATHOLOGICALLY **	19	49	44
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(19)	(49)	(44)
ADENOCARCINOMA, NOS			1 (2%)
SARCOMA, NOS		2 (4%)	
FIBROSARCOMA	1 (5%)		
RESPIRATORY SYSTEM			
#LUNG	(19)	(49)	(44)
ADENOCARCINOMA, NOS, METASTATIC			1 (2%)
ALVEOLAR/BRONCHIOLAR ADENOMA		1 (2%)	2 (5%)
ALVEOLAR/BRONCHIOLAR CARCINOMA		2 (4%)	
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(19)	(49)	(44)
MALIGNANT LYMPHOMA, NOS		3 (6%)	2 (5%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (5%)	2 (4%)	3 (7%)
UNDIFFERENTIATED LEUKEMIA			2 (5%)
#SPLEEN	(18)	(49)	(43)
HEMANGIOSARCOMA			2 (5%)
MALIGNANT LYMPHOMA, NOS	3 (17%)	1 (2%)	
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (6%)		
#LYMPH NODE	(17)	(45)	(38)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (6%)		
#MESENTERIC L. NODE	(17)	(45)	(38)
MALIGNANT LYMPHOMA, NOS		1 (2%)	1 (3%)
#LIVER	(19)	(49)	(44)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (5%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE B2 (CONTINUED)

	CONTROL (UNTR) 06-0355	LOW DOSE 06-0345	HIGH DOSE 06-0350
UNDIFFERENTIATED LEUKEMIA	1 (5%)		
#PEYERS PATCH	(18)	(47)	(39)
MALIGNANT LYMPHOMA, NOS		1 (2%)	
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(19)	(49)	(44)
NEOPLASM, NOS		1 (2%)	
HEPATOCELLULAR ADENOMA		3 (6%)	5 (11%)
HEPATOCELLULAR CARCINOMA	2 (11%)	2 (4%)	4 (9%)
HEMANGIOSARCOMA			1 (2%)
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#PITUITARY	(15)	(35)	(24)
ADENOMA, NOS	1 (7%)	5 (14%)	1 (4%)
#ADRENAL	(18)	(46)	(41)
CORTICAL CARCINOMA		1 (2%)	
PHEOCHROMOCYTOMA			1 (2%)
#ADRENAL/CAPSULE	(18)	(46)	(41)
ADENOMA, NOS		1 (2%)	
#THYROID	(17)	(42)	(38)
NEOPLASM, NOS			1 (3%)
FOLLICULAR-CELL ADENOMA	2 (12%)		
FOLLICULAR-CELL CARCINOMA		1 (2%)	
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(19)	(49)	(44)
ADENOMA, NOS	1 (5%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B2 (CONTINUED)

	CONTROL (UNTR) 06-0355	LOW DOSE 06-0345	HIGH DOSE 06-0350
#UTERUS	(17)	(47)	(38)
ENDOMETRIAL STROMAL POLYP	1 (6%)		
ENDOMETRIAL STROMAL SARCOMA		1 (2%)	
#OVARY	(17)	(39)	(38)
CYSTADENOMA, NOS	2 (12%)		
PAPILLARY CYSTADENOMA, NOS		1 (3%)	1 (3%)
SERTOLI-CELL TUMOR		1 (3%)	
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*HARDERIAN GLAND	(19)	(49)	(44)
ADENOMA, NOS		1 (2%)	
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	2	15	26
MORIBUND SACRIFICE	1	2	5
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	16	33	19
ANIMAL MISSING	1		

@ INCLUDES AUTOLYZED ANIMALS

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B2 (CONCLUDED)

	CONTROL (UNTR) 06-0355	LOW DOSE 06-0345	HIGH DOSE 06-0350
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	12	27	22
TOTAL PRIMARY TUMORS	18	31	27
TOTAL ANIMALS WITH BENIGN TUMORS	5	11	9
TOTAL BENIGN TUMORS	7	13	10
TOTAL ANIMALS WITH MALIGNANT TUMORS	9	16	13
TOTAL MALIGNANT TUMORS	11	17	16
TOTAL ANIMALS WITH SECONDARY TUMORS#			1
TOTAL SECONDARY TUMORS			1
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT		1	1
TOTAL UNCERTAIN TUMORS		1	1
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS
\# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

APPENDIX C

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN RATS TREATED WITH 2-CHLORO-p-PHENYLENEDIAMINE SULFATE

TABLE C1
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS
TREATED WITH 2-CHLORO-p-PHENYLENEDIAMINE SULFATE

	CONTROL (UNTR) 01-0355	LOW DOSE 01-0340	HIGH DOSE 01-0345
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	49	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	49	50
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(20)	(49)	(50)
NECROSIS, NOS			1 (2%)
RESPIRATORY SYSTEM			
#LUNG/BRONCHUS	(20)	(49)	(50)
BRONCHIECTASIS	2 (10%)	1 (2%)	
INFLAMMATION, NOS	1 (5%)		
#LUNG	(20)	(49)	(50)
INFLAMMATION, NOS	1 (5%)		1 (2%)
HYPERPLASIA, ALVEOLAR EPITHELIUM	1 (5%)		2 (4%)
HEMATOPOIETIC SYSTEM			
#SPLEEN	(20)	(49)	(50)
INFARCT, HEALED		1 (2%)	
HEMATOPOIESIS		5 (10%)	5 (10%)
#SPLENIC FOLLICLES	(20)	(49)	(50)
NECROSIS, NOS			1 (2%)
CIRCULATORY SYSTEM			
#HEART	(20)	(49)	(50)
INFLAMMATION, INTERSTITIAL		1 (2%)	
#MYOCARDIUM	(20)	(49)	(50)
INFLAMMATION, INTERSTITIAL		1 (2%)	
FIBROSIS	3 (15%)	1 (2%)	10 (20%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C1 (CONTINUED)

	CONTROL (UNTR) 01-0355	LOW DOSE 01-0340	HIGH DOSE 01-0345
DEGENERATION, NOS	5 (25%)	4 (8%)	2 (4%)
DIGESTIVE SYSTEM			
#LIVER	(20)	(49)	(50)
NECROSIS, FOCAL	1 (5%)		
METAMORPHOSIS FATTY		1 (2%)	2 (4%)
BASOPHILIC CYTO CHANGE	1 (5%)	2 (4%)	1 (2%)
ECSINOPHILIC CYTO CHANGE		1 (2%)	
#PANCREATIC ACINUS	(18)	(46)	(46)
ATROPHY, NOS	1 (6%)		
#STOMACH	(18)	(48)	(50)
ACANTHOSIS	1 (6%)		1 (2%)
#COLON	(18)	(46)	(49)
NEMATODIASIS	1 (6%)		
URINARY SYSTEM			
#KIDNEY	(20)	(49)	(50)
MINERALIZATION			1 (2%)
INFLAMMATION, NOS			1 (2%)
INFLAMMATION, CHRONIC		1 (2%)	
FIBROSIS, DIFFUSE		1 (2%)	33 (66%)
PERIVASCULITIS			1 (2%)
NEPHROPATHY	18 (90%)	47 (96%)	48 (96%)
DEGENERATION, CYSTIC		1 (2%)	
NEPHROSIS, NOS			1 (2%)
GLOMERULOSCLEROSIS, NOS		3 (6%)	
NECROSIS, NOS		1 (2%)	
HYPERTROPHY, NOS			1 (2%)
HYPERPLASIA, EPITHELIAL		14 (29%)	30 (60%)
#KIDNEY/PELVIS	(20)	(49)	(50)
HYPERPLASIA, EPITHELIAL		3 (6%)	
ENDOCRINE SYSTEM			
#ADRENAL CORTEX	(20)	(48)	(50)
HYPERTROPHY, NOS		1 (2%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C1 (CONTINUED)

	CONTROL (UNTR) 01-0355	LOW DOSE 01-0340	HIGH DOSE 01-0345
HYPERPLASIA, NOS	1 (5%)		1 (2%)
#ADRENAL MEDULLA	(20)	(48)	(50)
HYPERPLASIA, NODULAR			1 (2%)
HYPERPLASIA, NOS		2 (4%)	
#THYROID	(18)	(43)	(49)
FOLLICULAR CYST, NOS			2 (4%)
HYPERPLASIA, C-CELL	1 (6%)		1 (2%)
#PARATHYROID	(8)	(15)	(18)
HYPERPLASIA, NOS			1 (6%)
REPRODUCTIVE SYSTEM			
#PROSTATE	(19)	(46)	(48)
INFLAMMATION, NOS			1 (2%)
#TESTIS	(19)	(48)	(50)
MINERALIZATION	1 (5%)		2 (4%)
INFLAMMATION, NOS		1 (2%)	
ATROPHY, NOS	2 (11%)	5 (10%)	6 (12%)
HYPERPLASIA, INTERSTITIAL CELL			1 (2%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*EYE	(20)	(49)	(50)
CATARACT		1 (2%)	
*EYE/CORNEA	(20)	(49)	(50)
INFLAMMATION, NOS			1 (2%)
*HARDERIAN GLAND	(20)	(49)	(50)
INFLAMMATION, NOS			1 (2%)
MUSCULOSKELETAL SYSTEM			
NONE			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C1 (CONCLUDED)

	CONTROL (UNTR) 01-0355	LOW DOSE 01-0340	HIGH DOSE 01-0345
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
OMENTUM			
NECROSIS, FAT			1
SPECIAL MORPHOLOGY SUMMARY			
AUTO/NECROPSY/HISTO PERF	2		
AUTOLYSIS/NO NECROPSY		1	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C2
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS
TREATED WITH 2-CHLORO-p-PHENYLENEDIAMINE SULFATE

	CONTROL (UNTR) 02-0355	LOW DOSE 02-0340	HIGH DOSE 02-0345
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	49	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	49	49
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#TRACHEA	(5)	(48)	(47)
INFLAMMATION, NOS			1 (2%)
#LUNG/BRONCHUS	(20)	(49)	(49)
BRONCHIECTASIS			1 (2%)
INFLAMMATION, NOS	1 (5%)	2 (4%)	
#LUNG	(20)	(49)	(49)
INFLAMMATION, NOS		1 (2%)	
PNEUMONIA, CHRONIC MURINE		1 (2%)	
HYPERPLASIA, ALVEOLAR EPITHELIUM		1 (2%)	
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(19)	(48)	(46)
HISTIOCYTOSIS			1 (2%)
#SPLEEN	(20)	(48)	(49)
HEMATOPOIESIS	6 (30%)	16 (33%)	15 (31%)
CIRCULATORY SYSTEM			
#HEART	(20)	(49)	(49)
MINERALIZATION		1 (2%)	
#MYOCARDIUM	(20)	(49)	(49)
FIBROSIS		1 (2%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C2 (CONTINUED)

	CONTROL (UNTR) 02-0355	LOW DOSE 02-0340	HIGH DOSE 02-0345
DEGENERATION, NOS	2 (10%)	2 (4%)	2 (4%)
DIGESTIVE SYSTEM			
#LIVER	(20)	(49)	(49)
NECROSIS, FOCAL	1 (5%)	1 (2%)	1 (2%)
METAMORPHOSIS FATTY	2 (10%)	1 (2%)	
BASOPHILIC CYTO CHANGE	9 (45%)	10 (20%)	10 (20%)
ANGIECTASIS		1 (2%)	
*BILE DUCT	(20)	(49)	(49)
HYPERPLASIA, NOS		1 (2%)	
#STOMACH	(20)	(48)	(48)
HYPERKERATOSIS		1 (2%)	
ACANTHOSIS		2 (4%)	
#COLON	(20)	(47)	(49)
PARASITISM	1 (5%)		
URINARY SYSTEM			
#KIDNEY	(20)	(48)	(49)
MINERALIZATION		2 (4%)	
NEPHROPATHY	11 (55%)	37 (77%)	25 (51%)
HYPERPLASIA, EPITHELIAL		14 (29%)	8 (16%)
ENDOCRINE SYSTEM			
#ADRENAL CORTEX	(20)	(47)	(49)
HYPERTROPHY, FOCAL		1 (2%)	2 (4%)
#THYROID	(20)	(44)	(39)
FOLLICULAR CYST, NOS		1 (2%)	
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(49)	(49)
GALACTOCELE	4 (20%)	3 (6%)	1 (2%)
HYPERPLASIA, NOS	1 (5%)	3 (6%)	
#UTERUS	(20)	(48)	(49)
HYDROMETRA		1 (2%)	1 (2%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C2 (CONTINUED)

	CONTROL (UNTR) 02-0355	LOW DOSE 02-0340	HIGH DOSE 02-0345
INFLAMMATION, NOS		9 (19%)	7 (14%)
HYPERPLASIA, ADENOMATOUS		1 (2%)	
POLYP, INFLAMMATORY			1 (2%)
#UTERUS/ENDOMETRIUM	(20)	(48)	(49)
INFLAMMATION, NOS	5 (25%)		
HYPERPLASIA, NOS	1 (5%)	2 (4%)	3 (6%)
#OVARY/OVIDUCT	(20)	(48)	(49)
INFLAMMATION, NOS		1 (2%)	
#OVARY	(20)	(48)	(49)
CYST, NOS		1 (2%)	
INFLAMMATION, NOS		1 (2%)	3 (6%)
INFLAMMATION, NECROTIZING		1 (2%)	
DEGENERATION, CYSTIC			1 (2%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*EYE	(20)	(49)	(49)
CATARACT			1 (2%)
*EYE/RETINA	(20)	(49)	(49)
ATROPHY, NOS			1 (2%)
*HARDERIAN GLAND	(20)	(49)	(49)
INFLAMMATION, NOS			1 (2%)
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
OMENTUM			
MINERALIZATION	1		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C2 (CONCLUDED)

	CONTROL(UNTR) 02-0355	LOW DOSE 02-0340	HIGH DOSE 02-0345
NECROSIS, FAT	1		
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	1	2	7
AUTO/NECROPSY/HISTO PERF		1	
AUTOLYSIS/NO NECROPSY		1	1

* NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

APPENDIX D

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MICE TREATED WITH 2-CHLORO-p-PHENYLENEDIAMINE SULFATE

TABLE D1
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE
TREATED WITH 2-CHLORO-p-PHENYLENEDIAMINE SULFATE

	CONTROL (UNTR) 05-0355	LOW DOSE 05-0345	HIGH DOSE 05-0350
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING	2		
ANIMALS NECROPSIED	17	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY **	17	50	48
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(17)	(50)	(48)
INFLAMMATION, NOS		1 (2%)	
PNEUMONIA, CHRONIC MURINE		1 (2%)	
HEMATOPOIETIC SYSTEM			
#SPLEEN	(16)	(50)	(45)
HYPERPLASIA, NOS		1 (2%)	
HEMATOPOIESIS	2 (13%)	3 (6%)	1 (2%)
#MESENTERIC L. NODE	(14)	(46)	(42)
CONGESTION, NOS	1 (7%)		
HEMATOPOIESIS		1 (2%)	
CIRCULATORY SYSTEM			
*CARDIOVASCULAR SYSTE	(17)	(50)	(50)
PERIVASCULITIS		1 (2%)	
DIGESTIVE SYSTEM			
#LIVER	(17)	(49)	(46)
INFLAMMATION, NOS		1 (2%)	
INFLAMMATION, ACUTE		1 (2%)	
NECROSIS, FOCAL		11 (22%)	2 (4%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE D1 (CONTINUED)

	CONTROL (UNTR) 05-0355	LOW DOSE 05-0345	HIGH DOSE 05-0350
NECROSIS, COAGULATIVE	1 (6%)		
HYPERPLASIA, FOCAL		1 (2%)	1 (2%)
HEMATOPOIESIS			1 (2%)
*GALLBLADDER	(17)	(50)	(50)
CALCULUS, NOS		1 (2%)	
*BILE DUCT	(17)	(50)	(50)
HYPERPLASIA, NOS		2 (4%)	1 (2%)
#PANCREATIC ACINUS	(16)	(47)	(43)
ATROPHY, NOS	1 (6%)		
#STOMACH	(15)	(47)	(44)
ABSCESS, NOS		2 (4%)	
URINARY SYSTEM			
#KIDNEY	(16)	(50)	(47)
MINERALIZATION		1 (2%)	
ENDOCRINE SYSTEM			
#THYROID	(17)	(47)	(42)
FOLLICULAR CYST, NOS		1 (2%)	
HYPERPLASIA, FOLLICULAR-CELL	1 (6%)		
REPRODUCTIVE SYSTEM			
NONE			
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D1 (CONCLUDED)

	CONTROL (UNTR) 05-0355	LOW DOSE 05-0345	HIGH DOSE 05-0350
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(17)	(50)	(50)
HEMATOPOIESIS		1 (2%)	
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	4	17	14
ANIMAL MISSING/NO NECROPSY	2		
AUTO/NECROPSY/HISTO PERF			2
AUTO/NECROPSY/NO HISTO			2
AUTOLYSIS/NO NECROPSY	1		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D2
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE
TREATED WITH 2-CHLORO-p-PHENYLENEDIAMINE SULFATE

	CONTROL(UNTR) 06-0355	LOW DOSE 06-0345	HIGH DOSE 06-0350
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING	1		
ANIMALS NECROPSIED	19	49	44
ANIMALS EXAMINED HISTOPATHOLOGICALLY **	19	49	44
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(19)	(49)	(44)
CHOLESTEATOMA			1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(19)	(49)	(44)
INFLAMMATION, NOS		3 (6%)	
INFLAMMATION, FOCAL		1 (2%)	
HEMATOPOIETIC SYSTEM			
#SPLEEN	(18)	(49)	(43)
HYPERPLASIA, LYMPHOID		1 (2%)	
HEMATOPOIESIS	2 (11%)	4 (8%)	1 (2%)
#SPLENIC FOLLICLES	(18)	(49)	(43)
HYPERPLASIA, NOS		1 (2%)	
#MESENTERIC L. NODE	(17)	(45)	(38)
HEMATOPOIESIS		1 (2%)	1 (3%)
CIRCULATORY SYSTEM			
#HEART	(19)	(49)	(43)
AMYLOIDOSIS			1 (2%)
DIGESTIVE SYSTEM			
#LIVER	(19)	(49)	(44)
HYPERPLASIA, FOCAL		1 (2%)	1 (2%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE D2 (CONTINUED)

	CONTROL (UNTR) 06-0355	LOW DOSE 06-0345	HIGH DOSE 06-0350
HEMATOPOIESIS		1 (2%)	1 (2%)
*GALLBLADDER	(19)	(49)	(44)
INFLAMMATION, NOS	1 (5%)		
#PANCREAS	(18)	(44)	(40)
INFLAMMATION, CHRONIC		1 (2%)	
#PANCREATIC ACINUS	(18)	(44)	(40)
ATROPHY, NOS		1 (2%)	1 (3%)
#STOMACH	(17)	(48)	(40)
ABSCESS, NOS		2 (4%)	1 (3%)
#PEYERS PATCH	(18)	(47)	(39)
HYPERPLASIA, NOS	1 (6%)		
#DUODENUM	(18)	(47)	(39)
AMYLOIDOSIS			1 (3%)
#JEJUNUM	(18)	(47)	(39)
AMYLOIDOSIS			1 (3%)
#ILEUM	(18)	(47)	(39)
AMYLOIDOSIS			1 (3%)
URINARY SYSTEM			
#KIDNEY	(18)	(49)	(43)
INFLAMMATION, NOS			1 (2%)
GLOMERULOSCLEROSIS, NOS		1 (2%)	
AMYLOIDOSIS			1 (2%)
ENDOCRINE SYSTEM			
#ADRENAL/CAPSULE	(18)	(46)	(41)
HYPERPLASIA, NOS	3 (17%)	1 (2%)	
#ADRENAL CORTEX	(18)	(46)	(41)
HYPERTROPHY, FOCAL	1 (6%)		
#THYROID	(17)	(42)	(38)
FOLLICULAR CYST, NOS	1 (6%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D2 (CONTINUED)

	CONTROL (UNTR) 06-0355	LOW DOSE 06-0345	HIGH DOSE 06-0350
HYPERPLASIA, FOLLICULAR-CELL	3 (18%)		
REPRODUCTIVE SYSTEM			
#UTERUS	(17)	(47)	(38)
INFLAMMATION, NOS	1 (6%)		
#UTERUS/ENDOMETRIUM	(17)	(47)	(38)
HYPERPLASIA, NOS	5 (29%)	1 (2%)	1 (3%)
HYPERPLASIA, CYSTIC	1 (6%)	2 (4%)	
#OVARY/OVIDUCT	(17)	(47)	(38)
DEGENERATION, NOS			1 (3%)
#OVARY	(17)	(39)	(38)
MINERALIZATION		1 (3%)	
CYST, NOS		4 (10%)	
DEGENERATION, CYSTIC		1 (3%)	
AMYLOIDOSIS			1 (3%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(19)	(49)	(44)
AMYLOIDOSIS	1 (5%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D2 (CONCLUDED)

	CONTROL(UNTR) 06-0355	LOW DOSE 06-0345	HIGH DOSE 06-0350
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	1	11	15
ANIMAL MISSING/NO NECROPSY	1		
AUTO/NECROPSY/HISTO PERF		1	3
AUTOLYSIS/NO NECROPSY		1	6

\# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

Review of the Bioassay of 2-Chloro-*p*-phenylenediamine Sulfate*
for Carcinogenicity
by the Data Evaluation/Risk Assessment Subgroup
of the Clearinghouse on Environmental Carcinogens

April 26, 1978

The Clearinghouse on Environmental Carcinogens was established in May, 1976, in compliance with DHEW Committee Regulations and the Provisions of the Federal Advisory Committee Act. The purpose of the Clearinghouse is to advise the Director of the National Cancer Institute (NCI) on its bioassay program to identify and to evaluate chemical carcinogens in the environment to which humans may be exposed. The members of the Clearinghouse have been drawn from academia, industry, organized labor, public interest groups, State health officials, and quasi-public health and research organizations. Members have been selected on the basis of their experience in carcinogenesis or related fields and, collectively, provide expertise in chemistry, biochemistry, biostatistics, toxicology, pathology, and epidemiology. Representatives of various Governmental agencies participate as ad hoc members. The Data Evaluation/ Risk Assessment Subgroup of the Clearinghouse is charged with the responsibility of providing a peer review of reports prepared on NCI-sponsored bioassays of chemicals studied for carcinogenicity. It is in this context that the below critique is given on the bioassay of 2-Chloro-*p*-phenylene-diamine Sulfate for carcinogenicity.

The primary reviewer agreed that the study did not provide statistically significant evidence of the carcino-genicity of 2-Chloro-*p*-phenylenediamine Sulfate in rats or mice. She said that it was unclear as to how the tested material related to the commercially-used dye. She questioned whether the route of exposure was relevant to the human situation. The primary reviewer noted that the treatment of the high dose mice had to be terminated early due to excessive mortality and also the unexplained toxicity, manifested as alopecia, in both treated and control animals. She commented on the increased incidence of transitional-cell tumors of the urinary bladder found in treated rats but absent in the matched controls. The primary reviewer suggested that a statement was needed in the summary with respect to the incidence of these lesions in historic control animals. A Program staff pathologist commented

that hyperplasia of the renal pelvis is a relatively rare lesion and is usually associated with bladder carcinogens.

A motion was approved unanimously that the report on the bioassay of 2-Chloro-*p*-phenylenediamine Sulfate be accepted.

Members present were:

Michael Shimkin (Acting Chairman), University of California at San Diego
Joseph Highland, Environmental Defense Fund
George Roush, Jr., Monsanto Company
Louise Strong, University of Texas Health Sciences Center
John Weisburger, American Health Foundation

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* Subsequent to this review, changes may have been made in the bioassay report either as a result of the review or other reasons. Thus, certain comments and criticisms reflected in the review may no longer be appropriate.

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